No. 3.— *The Spermatogenesis of Phrynotettix magnus, with special Reference to Synapsis and the Individuality of the Chromosomes.*

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TABLE OF CONTENTS.

I. INTRODUCTION.

A. Outline of the Problems.

Two of the most important subjects which have claimed the attention of cytologists for many years are the two named in the subtitle of this paper. Every species of animals and plants is thought to have a definite number of chromosomes, which is characteristic for the species. In the process of maturation this typical, or diploid, number becomes reduced so that each functional gamete contains only half that number, the haploid number. It is generally believed that the process of reduction is initiated by a pairing of the chromosomes in the prophase of the first maturation division. It is also generally admitted that of the two chromosomes which united to form a single pair, one has been derived from the maternal, and the other from the paternal ancestor, and that these become separated again at one of the two maturation divisions. But there has been a considerable amount of disagreement as to how the pairing of the chromosomes takes place, and also differences of opinion as to which of the two maturation divisions results in their separation.

As to the process by which the pairing of chromosomes is accom-

plished, the two opposing views that have been most widely discussed are: — (1) that homologous chromosomes unite side-by-side (parasynapsis), or (2) that they unite end-to-end (telosynapsis). The adoption of either view, however, involves the very important assumption that there is a continuity between the chromosomes that appear in the earlier divisions and those that conjugate. Doubt has been expressed by some writers as to the existence of any such continuity, or individuality, of the chromosomes, and the question is regarded as one that is still unsettled. Many geneticists, on the other hand, are readily inclined to correlate the behavior of the chromosomes in maturation with the behavior of Mendelian factors in heredity. And in some cases an organization of the individual chromosomes has been assumed of such a nature that a definite portion or region of a chromosome is concerned with the transmission of a particular factor. Such assumptions call for an analysis of the individual chromosomes to determine their inner constitution or architecture.

The author of the present study has sought to throw light on all these problems. That as to how synapsis takes place was the first considered; it was taken up from the standpoint of the origin and constitution of the chromosomes of the first spermatocytes. Early in the work it was found that the only method by which conclusive results could be obtained was that of following the history of individual chromosomes. Owing to the favorableness of the material, at least three chromosome-pairs were found that possessed individual peculiarities by which they could be recognized through all stages from the growth-period to their division in the first spermatocyte mitosis. Pursuit of this method naturally led to a consideration of the problem of the individuality of the chromosomes, and it was found to be possible to recognize one pair of chromosomes at all stages from spermatognia to spermatids. A further study of chromosome-individuality led to the interesting discovery that each chromosome has a definite organization, or architecture, which appears at the same stages in all the animals studied.

In the following description, I have not followed the usual method of adhering to the chronological sequence of events, but have adopted the order in which the problems presented themselves. I believe I have been able through a study of this material to demonstrate that in Phrynotettix (1) parasynapsis occurs, (2) usually the first maturation is equational, (3) each chromosome preserves its individuality throughout the spermatogenic cell-generations, and (4) at least certain chromosomes, and probably all, have a recognizably constant organization.

B. Material and Acknowledgments.

Phrynotettix magnus belongs to the subfamily Oedipodinae of the orthopteran family Acrididae. The specimens that furnished the basis for this investigation were collected in 1907 near the Santa Rita Mountains of southern Arizona, by a collecting party from the University of Kansas. The testes were dissected out and fixed in Flemming's stronger solution. Sections were cut 6–12 micra thick and stained either by Heidenhain's iron-haematoxylin, or by Flemming's tricolor, method. Material from thirteen animals was available and consisted partly of the slides used by Miss Pinney as the basis of her paper of 1908, partly of other slides prepared in Dr. McClung's laboratory, and lastly of material sectioned and stained by the writer.

The work was begun in 1911 at the University of Kansas under the direction of Prof. C. E. McClung, to whom I am indebted for the material used and for advice and kindly interest throughout. The greater part of the work was done at Harvard University during the years 1912–1915 under the direction of Prof. E. L. Mark, to whom I owe my warmest thanks for valuable criticism and suggestions and for sympathetic interest at all times. I am also indebted to Miss Eleanor Carothers, formerly a fellow student, for some collaboration, especially with reference to the so-called "plasmosomes."

II. OBSERVATIONS.

A. Outline of spermatogenesis: Nomenclature.

a. Introductory.

There is some confusion in the literature on maturation in regard to the use of the terms applied to the various steps and processes in the history of germ-cells undergoing development into gametes. This is due in part to differences in the details of the processes in the various forms investigated, and in part to different interpretations of similar stages by different authors. It therefore seems necessary, or at least expedient, to explain the terms that one wishes to use in description. A brief outline of acridian spermatogenesis follows, in connection with which the nomenclature employed will be explained. In addition,

enough of the peculiarities of Phrynotettix will be described to render clear any new terms made necessary by them.

Wilcox (**'94**) and Davis (**'08**) have both given detailed descriptions of the structure of the acridian testis and have given figures or diagrams to show the topography of the follicles of which the testes are composed. It will therefore be unnecessary to reproduce such figures and descriptions here.

b. Outline of successive Stages.

1. *Spermatogonia.*— The spermatogonia of Phrynotettix (Plates **1**, **2**, fig. 1–20) behave in a manner typical for the Acrididae as described especially by Sutton (**'00**), and by Davis (**'08**). As Pinney (**'08**) has shown, there are 23 chromosomes, of which 22 can be arranged in pairs, leaving an odd one, the accessory chromosome (McClung, **'99**), or monosome (Montgomery, **'06**). The paired chromosomes may be referred to as the autosomes (Montgomery, **'06**). All the divisions of the spermatogonia are mitotic and are usually considered as equivalent to somatic mitoses. A detailed account of these divisions is given on pages 87–90.

2. *Primary spermatocytes.*— The daughter cells produced at the final spermatogonial division, as is well known, are characterized, among other things, by the growth-period and by the formation of the reduced, or haploid, number of chromosomes. For distinguishing the different stages in the prophases, Davis (**'08**) employed a non-descriptive method, designating successive stages by the successive letters of the alphabet. Here it seems advisable to use largely the terminology introduced by Winniwarter and by Grégoire.

The telophase of the last spermatogonial division embraces a series of processes similar to those in the telophases of the earlier spermatogonial divisions. Following the telophase, a series of changes takes place which results eventually in the formation of fine single threads. This fine-thread stage may be called the *leptotene* stage (Winniwarter, **'00**). Between the telophase and the leptotene stages occur changes which are of the utmost importance in any attempt to solve the problems of synapsis and the individuality of the chromosomes. These stages may be called the *preleptotene* stages (Grégoire, **'07**). There may be distinguished an earlier (Plate **2**, fig. 23, 24) and a later (Plate **3**, fig. 25–27) preleptotene stage.

When the leptotene threads are first formed, they seem to be greatly

tangled and lack definite arrangement. This condition, which may be called early leptotene (fig. 28), is followed by a later leptotene (fig. 29), in which the threads become oriented with one end attached at one side (the polar side) of the nucleus. Soon there appear among the single threads others which are double and twice the width of the single ones. The proportionate number of double threads gradually increases until all the threads appear double. The stage during which the doubling takes place (fig. 30, 31) is the *zygotene* stage of Grégoire (**'07**). When all the threads have become double the *pachytene* stage (Winniwarter, **'00**) has been reached (Plate **3**, fig. 32–34). This term continues to be applicable throughout the relatively long growth-period, and until the spireme [1] breaks up into the haploid number of segments, which become tetrads. The number of pachytene threads seems to be much less than that of the leptotene threads.

The stages characterized by the appearance of separate segments of the spireme may be designated by the term *diplotene* of Winniwarter (**'00**). This term is used for the sake of consistency with the others employed, although the conditions in Phrynotettix differ somewhat from those described by Winniwarter for mammals. He describes the longitudinal split as disappearing in the pachytene stages, on account of the threads becoming twisted, and reappearing in the diplotene stage. In Phrynotettix the longitudinal split remains visible and little or no twisting occurs.

Soon after becoming independent, a second longitudinal split occurs in the spireme segments at right angles to the first, thus forming typical tetrads, each composed of four *chromatids* (McClung, **'00**). The first longitudinal split, which persists from the pachytene stage, may be called the *primary split*, and the one at right angles to it may be called the *secondary split*. From the time of their formation until the succeeding metaphase, the tetrads undergo a gradual shortening and thickening. During this period they pass through the well-known figures, X's, K's, 8's, rings and crosses (Plate **3**, fig. 38). The stage during which these changes occur is frequently referred to as the *diakinesis* stage (Häcker, **'95**[a]), but it may be simpler to call it the *postspireme* stage (Grégoire, **'07**), or the tetrad stage.[2]

The postspireme stages end with the establishment of the tetrad-

[1] The term spireme will be used to embrace the stages included under the names leptotene, zygotene, and pachytene without, however, implying anything as to the existence of a continuous thread.

[2] I have avoided the use of the term, prophase, in connection with the postspireme stages because it might properly be applied to the whole series of stages from the preleptotene to the metaphase.

chromosomes upon the mitotic spindle of the first spermatocyte division. The number thus appearing on the spindle is twelve (Plate 4, fig. 39). One of them is the accessory chromosome, which is a dyad and passes to one pole undivided (Plate 4, fig. 41, *X*). The eleven tetrads represent the other twenty-two spermatogonial chromosomes arranged in pairs. One daughter cell of each spermatocyte receives eleven dyads and the other receives twelve, the additional one in the latter case being the accessory chromosome.

In the anaphase all the chromosomes appear as V's, thus showing their dyad constitution (fig. 42 and 43). Before this, in the metaphase, the separate chromatids are not discernible, but early in the anaphase they separate from each other at the end opposite that which is attached to the spindle-fiber, in this way giving rise to the V-shaped figures. The V-shaped arrangement persists until the metaphase of the succeeding division is reached.

3. *Secondary spermatocytes.*— The secondary spermatocytes present only a short resting stage. For this stage, between the formation of the secondary spermatocytes and their division, we may employ the term *interkinesis* (intercinèse) proposed by Grégoire (**'05**). The extent of diffusion reached by the dyads in interkinesis is much greater than that usually described for this stage, as will be seen from figures 46 and 47 (Plate 4). The dyads reappear however, in the same orientation and relative positions that they had before diffusion.

In the metaphase the dyads show the same double structure that they did in the anaphase of the immediately preceding division (Plate 5, fig. 50–52). The two monads composing each dyad are separated from each other in the metaphase, and in the anaphase are carried to the poles of the spindle (fig. 54). Half of the secondary spermatocytes show in the plates of the metaphase eleven chromosomes and the other half twelve chromosomes, as was to have been expected owing to the non-division of the monosome in the division of the primary spermatocytes. Figure 50 (Plate 5) shows eleven and figure 51 shows twelve chromosomes.

The term *reductional* will be used to designate that one of the two maturation divisions which results in the separation of the chromosomes that conjugated in synapsis. Correspondingly the term *equational* will be applied to the division in which the halves of whole chromosomes are separated. Employing the terminology of Korschelt und Heider (**'03**), we may use the terms *prereduction* when the first maturation division is reductional, and *postreduction* when the second division is reductional.

4. *Spermatids.*— The spermatids, daughter cells of the secondary spermatocytes, undergo gradual transformation, without further division, into the mature spermatozoa. Their chromosomes undergo dissolution, having, however, first formed a network not unlike that found in the telophase of ordinary mitoses.

5. *Spermatozoa.*— This term is used, as usual, to designate the functional male gametes — the end products of all the preceding processes.

c. Additional Features.

In Phrynotettix, as pointed out by Pinney (**'08**), there appear in many of the stages of spermatogenesis condensed and deeply staining granules at the ends of the chromosomes. These granules are recognizable in the stages where the greater part of the chromatin is extended or diffuse, so that their density, contrasted with that of the rest of the chromatin, brings them into view. Figures 8, 10, 12 (Plate **1**) and 14–20 (Plate **2**) show them for the spermatogonia; figures 28–38 (Plate **3**) for the primary spermatocytes; figures 45–48 (Plate **4**) for the interkinesis stage, and figure 55 (Plate **5**), for the spermatids. These granules appear at that end of each chromosome — including the accessory — to which the spindle-fiber attaches. They were named accordingly by Miss Pinney *polar granules.* In the case of certain chromosomes, as noted by her, similar granules also occur at the end of the chromosome opposite that to which the spindle-fiber attaches. The chromosomes are thus seen to exhibit polarity and it will therefore be convenient to designate the two ends by different terms. In the absence of better terms, I shall call the end to which the spindle-fiber attaches the proximal or synaptic end, and the opposite one the distal end.

At various stages there is a tendency for some of the polar granules to fuse together, as noted by Pinney **'08**, forming what I shall call *composite granules.* These are to be seen in the telophase and prophase of the spermatogonia (Plate **1**, fig. 12; and Plate **2**, fig. 14, 16), in the spireme stages of the primary spermatocytes (Plate **3**, fig. 33–36), and even in the connective-tissue nuclei (Plate **9**, fig. 108–110). They are particularly noticeable in the pachytene stages, for during that period quite large masses of chromatin may form by the coalescence of a number of these polar granules. The number of granules making up a composite granule is variable, but may usually be determined by the number of spireme threads attached to it. These

sometimes radiate out from the composite granule like the spokes of a wheel from its hub. Such a stage corresponds to the *bouquet* stage of Eisen ('00). At the end of the pachytene stage the granules composing the composites separate out again, apparently without having changed their identity (Plate 3, fig. 35–37).

The tendency of the polar granules to remain on one side of the nucleus may be interpreted as evidence of a somewhat persistent polarity of the nucleus as a whole. It will therefore be convenient to speak of that region of the nucleus where the majority of the polar granules are congregated as the *proximal* pole, and the opposite side as the *distal* pole, of the nucleus.

In my description of the leptotene and zygotene stages it will have been noticed that no mention is made of the contraction, or *synezesis*, stage (McClung '05). Such a phenomenon has not appeared in my material and, as has been claimed by McClung ('00, '05), Davis ('08) and others, is probably not normal in the Orthoptera.

I shall use the term synapsis in the same sense in which it was originally used by Moore ('95), that is, to indicate the process of coupling or conjugation of the chromosomes of the last spermatogonia to form those of the first spermatocyte. Following Wilson ('09), I shall use *parasynapsis* to denote side-by-side conjugation, and *telosynapsis* to denote end-to-end conjugation.

For the purpose of determining more accurately the history of the changes undergone by the chromatin through the successive stages outlined above, three individual autosome-pairs have been selected for detailed study. To distinguish them from the other autosomes, I shall call them the *selected* chromosomes.

B. Synapsis.

a. The Postspireme Stages.

Of the various methods by which the diploid series of chromosomes could unite in pairs to form the haploid, or reduced, series, the two which have been more frequently defended are: — (1) that by which the members of each pair unite end-to-end (telosynapsis), and (2) that by which they unite side-by-side (parasynapsis). Evidence in favor of both methods has been gained from observations on orthopteran material. The writer, without prejudice in favor of either view, undertook to discover which of these processes occurs in Phrynotettix.

Efforts were first directed to a study of the postspireme stages in the hope of discovering how the segments of the pachytene spireme became the tetrads exhibiting the shapes of V's, X's, 8's, crosses and rings. Such a variety of shapes and forms presented themselves at any one of the tetrad stages, however, that it was impossible to decide which were the more primitive and which the derived forms. Figure 38, *a–g* (Plate 3), for example, shows some of the different shapes of tetrads seen in a single stage and, indeed, in the same cyst. The only method that seemed to offer a means of securing decisive evidence on the problem was that of following the history of individual chromosome-pairs through a large number of stages. For this purpose it was necessary to find pairs which possessed individual characteristics by which they could be recognized in all the stages concerned. Fortunately, at least three pairs were found which fulfilled these requirements. For convenience in description they have been designated "*A*," "*B*," and "*C*."

1. *Chromosome-pair A.*— This element was first distinguished in the pachytene stage, where it is a very deeply stained spireme segment. Examples of it are shown in figures 56 and 57, (Plate 5). Its differential staining property is so marked and constant that it can be recognized by this character alone up to the later postspireme stages. But there is an additional means of identification. Like most of the pachytene threads, this one normally makes a loop the two ends of which approach to, or attach at, the proximal pole of the nucleus (Plate 5, fig. 56). One or both ends may become free from entanglements, but more frequently only one. In the latter case the free end, or if both ends are free, one of them, is nearly always terminated by two knobs, of which one is usually larger and less deeply stained than the other (Plate 5, fig. 57*g*; Plate 10, fig. 113). These knobs, I believe, may be identified as the polar granules described by Miss Pinney ('08). But in this instance, as shown by numerous observations, the more prominent granules occur at the distal end of the chromosome instead of the proximal end, where they are found on the majority of the other chromosomes. That the expanded condition of one of the granules furnishes a means of identification, will be apparent from an examination of figure 62 (Plate 6).

As an exceptional occurrence these two terminal granules may be equal in size, neither one being expanded. In order to test the relative frequency of these two conditions, some counts were made and tabulated for both the spireme and postspireme stages, as follows: —

Stage	Total	Expanded	Not expanded	% Expanded
Spireme	111	94	7	93.06
Postspireme	162	146	16	90.12
Both stages	273	240	23	91.26

It will be seen from this table that approximately 90% (examples counted at random) have one of the granules in the expanded condition. In the postspireme stages this peculiarity appears less like an expanded single granule than as a group of closely associated small granules, typically three in number. This condition will be discussed more fully in another place (p. 112). In both the spireme and the postspireme stages the modified polar granule furnishes a ready means of identification of chromosome-pair *A*, especially when its staining qualities, already described, are taken into consideration. The constant relative size of *A* in the tetrad stages is also a help in identification.

Figure 62 (Plate 6) indicates clearly the processes by which the spireme loop becomes first transformed into a typical tetrad, and then condensed to a metaphase chromosome. From the zygotene stage onward, there is a gradual shortening of the spireme loops or segments. The later stages of this process are to be seen in figure 62. Throughout the pachytene stage the spireme loops exhibit a median longitudinal cleft, usually referred to as the longitudinal split. I shall call this the primary longitudinal split. Occasionally paired granules, or chromomeres, appear to be fused together, but as a general rule, the split is continuous throughout the length of the loop. In my opinion this so-called longitudinal split is really the space between two spireme (leptotene) threads which have conjugated side-by-side. Further evidence for this belief will be presented later.

Figure 62, *c*, indicates the first step in the process of forming the four chromatids of the tetrad. A second longitudinal split, at right angles to the first or primary split, begins at the proximal end (upper end in the figures) of the free spireme segment (fig. 62, *c*) and gradually proceeds toward the distal (lower) end (fig. 62, *c–e*). It will be seen from these figures that as the separation produced by the secondary split proceeds distally, the separated chromatids at the same time reunite along the plane of the primary split. The separation due to the secondary split gradually increases until the diverging pairs of chromatids extend in opposite directions, thus forming a rod-like element the two ends of which correspond to the proximal pole of the

original spireme segment, and its middle point to the distal pole. The rod-shaped tetrad becomes oriented in the spindle of the first maturation division with its long axis parallel to the spindle-axis, and at metaphase separates in the middle. In other words, the plane of the secondary split becomes the plane of the first maturation division, which is therefore equational. If now we may assume that the longitudinally split spireme segment has represented a pair of chromatin-threads which had conjugated side-by-side throughout their length, the plane of the primary split must be the plane of the reductional division, which becomes effective in the second spermatocyte mitosis.

The tetrad *A* also forms rings, as shown in figure 62, *j*, *k*, *l* (Plate 6). I have not been able to trace these rings into the metaphase to determine their orientation on the spindle, and furthermore I am quite uncertain whether the ring shape persists as far as the metaphase. Most of the metaphase figures show one tetrad in the form of a rod with its axis parallel to the spindle-axis, and with a constriction in the middle, as shown in figure 62, *i* and figure 79, *A* (Plate 7). Sometimes two or more rod-shaped tetrads are to be seen in the same spindle and with the same orientation. However, one of them is always in a more advanced stage of division than the others, and I have been inclined to identify this precocious one with tetrad *A*. Figure 62, *c–i*, indicates that such a conclusion is justified. Since the straight-rod condition is so characteristic of the metaphase, it may be that the rings also become transformed into straight rods by the time the metaphase is reached.

The rings seem to have been formed either by a failure of the proximal ends to separate during the formation of the secondary longitudinal split, or by a secondary union of these ends, *i. e.* after the split had begun. For example, if a tetrad in the condition of figure 62, *c*, has the secondary split completed without the separation of the proximal ends, a ring would result. So also would a ring be formed by a secondary union of the two proximal ends of a stage such as is seen in figure 62, *d* or *e*. In either event the region within the ring would represent the space formed as a result of the secondary longitudinal split. If the chromatids should now begin to separate at the proximal end along the plane of the primary split, as seems to be indicated in figure 62, *k* and *l*, and if this process should be continued until a metaphase chromosome such as that shown in *i* is produced, there is every reason to believe that it would result in a separation of the original conjugants of the pair, and therefore constitute a reductional division. On the other hand, it is possible that the separation along

the plane of the primary split is never completed, but that the chromatids again become separated at the proximal end, assuming the forms shown at *f* to *i*, figure 62, and that the first division is therefore always equational. However, the possibility of an occasional reductional division as a result of the ring-formation must be taken into consideration.

2. *Chromosome-pair B.* Figure 63 (Plate 6) presents a series of stages for *B* corresponding to those in figure 62 for *A*. This series of stages of *B* supports the conclusions reached from a study of *A* in regard to: — (1) a probable parallel association in the pachytene stages of pairs of threads, each representing individual chromosomes; (2) the formation of the tetrad by, first, a separation along the plane of conjugation (*i. e.*, the primary longitudinal split) and, secondly, by a splitting of each of the original conjugants (the secondary longitudinal split); and (3), as a result, an equational division of the tetrads at the first division.

This chromosome-pair (*B*) is characterized by the presence of large and well-marked polar granules at both ends and by a similar large granule not far from the middle, though always somewhat nearer the distal end. Leaving aside the formation of rings, the chief difference in behavior between *A* and *B* is that in the former the plane along which the greatest separation takes place before metaphase is that of the secondary longitudinal split, while in the latter the greatest separation takes place along the plane of the primary split. This results in *A* becoming extended in the direction of the spindle-axis, as already described, while *B* becomes extended at right angles to this axis. In the latter case the separation along the plane of the primary split does not become complete at the expense of the separation along the plane of the secondary split, but the latter separation persists for a short distance, giving rise to a cross with unequal arms (fig. 63, *g*, *h*). The short arms terminate in the proximal or synaptic ends of the chromatids, while the longer arms terminate in the distal ends.

However, these differences in behavior between *A* and *B* are not fundamental, since the final result, an equational division, is the same in both cases. But they are indications of the individual peculiarities of these elements. It should also be pointed out that such differences could easily be misinterpreted, if only parts of the histories of the pairs were known.

It is important to note that the drawings of the series shown in figure 63 were all taken from sections of a single testis. In searching for the same element in other individuals, I was surprised to find the

condition shown in figure 64, *a–h*. In this series are found the same differentiating characters that have already been described for *B*, except that one member of the large pair of granules at the distal end is lacking. In other words, we have to do here with a pair, composed of unequal elements, which differs from its homologue in another individual, composed of equal elements, by the absence of a definite part of one of the components. Examination of all the thirteen individuals demonstrated that eleven of them possessed this second or unequal type, while only two showed the equal type.

If there could have been any doubt about the sequence of events in the transformation of a spireme segment into a tetrad and the subsequent equational division in the case of chromosome-pair *A* or the equal type of *B*, the behavior of this unequal type of *B*, as shown in figure 64, must certainly make the subject clear. In this instance, on account of the difference between the two members, it is possible to identify them in such a way that there can be no question as to the two planes of longitudinal splitting. The figures have in all cases been made with great care with the aid of a camera lucida and are faithful reproductions of the conditions seen under the microscope so far as they can be represented by the method of reproduction used.

In the early stages of the transformation of the spireme segments into tetrads, the separate chromatids are not distinguishable throughout the whole length of the segment. This is due in part to a closer association of the chromatids and in part to the fact that one of the longitudinal splits becomes more pronounced at one end and the other split at the other end of the tetrad. Somewhere between the ends, therefore, there is a crossing or apparent chiasma. At the point of the crossing the chromatids at first appear to be fused together (figs. 63, *d* and 64, *d*). Very soon, however, the confusion disappears, the chromatids become distinct, and their relationships easily discernible, as shown in figures 63, *e*, and 64, *e*. In both these cases the wide separation at the proximal end has been along the plane of the secondary longitudinal split, and that at the distal end along the plane of the primary split. The resulting crossing, or apparent chiasma, is a perfectly normal and natural result of these processes and indicates nothing in the way of a breaking or recombining of the parts of chromatids.

3. *Chromosome-pair C.*— Figure 65 (Plate 6) shows one form of the third of the three selected chromosome-pairs. In this case the two components are very unequal in size, one of them possessing a very large, condensed mass, or granule, of chromatin at its distal end,

while the other has none. It will be noticed (fig. 65, *b*, *c*) that the details of the two components are quite homologous up to the large distal granule, and that the point of attachment of this large granule seems to correspond to the distal end of the smaller component. These considerations would lead us to suppose that here, too, as in the unequal type of *B*, the difference between the members of the pair may be due to the loss by one of them of a definite part possessed by the other. In this case, however, no such equal pair has been found as occurs in *B* when both members possess the part in question. The side-by-side association of the members of this pair is as evident as it was for *A* and *B* and the relations of the two longitudinal splits are the same.

In regard to the mode of distribution of tetrad parts in the first maturation division, however, we meet in this case a curious exception to the general rule. This pair divides equationally, as shown in figure 65, *h–j;* but it sometimes divides reductionally as shown at *k–m*, same figure. From casual inspection it appeared that the division occurred as frequently in the one manner as in the other. But in order to test the relative frequencies of the two methods, 928 cases chosen at random were counted and it was found that of this number 472, or 50.8%, were in process of reductional division, while 456, or 49.2%, were dividing equationally. It would seem from these counts that the method by which the tetrad divides is a matter of mere chance. This is the more apparent when we take into consideration the fact, brought out by extended observations, that the two methods occurred side-by-side in the same cysts. It may be that the shape or position of the tetrad when it is first brought under the influence of the mitotic spindle determines the mode of division.

The fact that this unequal pair divided in the first division reductionally a part of the time made it possible to study the distribution of the two conjugants with reference to the accessory chromosome, which goes to one pole undivided. It was soon found that either member of the pair could accompany the accessory into the secondary spermatocytes. Consequently counts were made to determine whether the two kinds of distribution occurred with anything like equal frequency. Out of 421 cases counted at random 216, or 51.3%, were found to show the larger member going to the same pole as the accessory (Plate **10**, fig. 121, *C*), while in 205, or 48.7%, of the cases the smaller member was going with the accessory to the same pole (fig. 120, *C*). These results seem to furnish a good example of chance distribution of chromosomes at maturation.

The behavior of these three selected chromosome-pairs, as described

above in detail, seems to me to establish very definitely that the association of paired chromosomes in the pachytene stages is one in which the members lie side-by-side throughout their entire length, and therefore exhibit *parasynapsis*. I should further add that while I have not singled out any other members of the complex for individual study, a careful analysis of the other spireme segments and the derivative tetrads indicates that the condition of parasynapsis is realized for the entire series. I was thus able to analyze the stages of the complex as a whole after following the history of the selected individual pairs, whereas previously I was unable to reach a definite conclusion.

As to the method of division in the first spermatocytes, the evidence presented indicates that *B*, always, and *A*, in most cases, divide equationally, while *C* divides either reductionally or equationally and with equal frequency by each method. My study of the other tetrads leads me to think that, as a general rule, they divide equationally in the first division. Where the first division is equational the second is regarded as reductional, and we therefore have postreduction. The general rule has its exceptions, however, as already noted in the case of *C* and possibly sometimes in the case of *A*.

b. The Conjugation of Chromosomes.

1. *The formation of leptotene threads.*— The evidence for parasynapsis derived from a study of the postspireme stages, as presented in the preceding paragraphs, has not embraced the actual process of conjugation; and it therefore remains to be demonstrated that a side-by-side conjugation does take place. But it is even more important to show that the conjugants are actually chromosomes, the morphological descendants of the telophase chromosomes of the final spermatogonial division. Figure 21 (Plate 2) shows a side view and figure 22 a transverse (optical) section through the chromosomes of cells nearing the end of the telophase of the last spermatogonial division. The side view shows the chromosomes already partly diffused, but each one occupies a definite territory, so that there is no question as to their persistent individuality, except for the coalescence of some of the polar granules. But, as I shall point out later, the polar granules do not necessarily lose their identity when they unite into the compound masses. The optical section, figure 22, shows even more plainly the persistent individuality of the chromosomes up to this

point, for there still can be seen the remnants of the vesicular walls which surrounded each chromosome in the earlier telophase.

There are 21 of these chromatin-masses, or "blocs" (Janssens, '01), shown in this optical section, and that is sufficiently close to the total number, 23, to indicate that all the chromosomes are still independent, except for the union at the polar ends, as already mentioned.

In figure 21 it will be noticed that the diffusing chromatin is disposed roughly in the form of spirals. Figures 23 to 29 indicate what becomes of these spirals in the "blocs" of chromatin. I am not quite sure of the exact succession of stages here, but believe they are about as shown in the successive figures. It is possible that figures 23 and 24 — which are side view and optical section, respectively, of the same stage — are no earlier than the stages shown in figure 25 (Plate 3). However that may be, the evidence seems to indicate that each of the blocs at stages such as those shown in figures 21 and 22 gives rise to a single fine thread, at first much coiled but later much elongated.

The side view shown in figure 23 is at a stage the casual examination of which might lead one to suppose that the chromatin was in a hopeless tangle without any definite arrangement whatever. But careful focussing and patient study revealed what I have tried to show in figure 23, viz., that the chromatin is still disposed, for the most part, in separate blocs, but that a very much coiled and convoluted thread is forming within each one of these territories. Some have unraveled to a considerable extent, and have become extended in various directions through the nuclear sap. But each seems to be a continuous thread, despite some tendency for the ragged edges at times to be connected with adjacent threads. In the optical section of this stage (fig. 24) it will be seen that the blocs have remained in place and separate from each other for the most part, though some anastomosis of the linin fibers has taken place at the periphery of the blocs. On the other hand, there are still some remnants of the previously existing vesicular walls, as shown in the left side of the figure. When one focusses up and down on such a cell, it is possible to follow in some cases the thread which is differentiating out of the net-like structure of each bloc, but in optical section the reticulum is more apparent than the continuous thread. The section shows nineteen blocs, which number is not far from the somatic number of chromosomes (23). Figures 23 and 24 represent what I have called the preleptotene stage.

At the stage shown in figure 25 (Plate 3), which I believe to be slightly more advanced than the one in figures 23 and 24, the amount of anastomosis between adjacent chromatic elements seems consid-

erably greater than in the stage last described. The anastomosis is to be seen more particularly at the sides of figure 25. Through the middle of this figure the individual spiral threads seem to be more easily distinguishable, and I am inclined to believe that the two which stain more deeply than the others are the members of the *A* pair of chromosomes. The stages including and following this reticular stage are hard to represent in a drawing of the kind employed, owing to the difficulty of portraying in their natural relations the parts seen at different planes of focus. Careful study has always convinced me, however, that the uncoiling and elongating threads are single, continuous, and not united into an indiscriminate network. I have selected in figures 26 and 27 views favorable for drawing where some of the threads, at least, are definitely separate and continuous across the diameter of the nucleus.

At the stage represented in figure 28 (Plate 3) the unwinding of the coiled threads has been completed, but the threads have as yet no definite orientation. At the somewhat later leptotene stage shown in figure 29 the threads are finer and less homogenous than in the earlier stage, the substance of the thread seeming to have become more distinctly differentiated into a linin fiber and chromatic granules, the latter scattered at irregular intervals along the fiber. Moreover, in this later stage the threads appear to be definitely oriented, with one end attached at the proximal pole of the nucleus. The threads then take a course through the center of the nucleus or near its periphery, extending wholly or partly across and then turning back with a wide curve.

2. *The zygotene stages.*— In figure 30 (Plate 3) some of the threads are double, others are single, and it would be difficult to decide from a casual examination of this stage alone whether or not the double threads had arisen by a splitting of the single ones. In the case of one or two of the double threads, however, as may be seen at the left side of the nucleus, the double condition does not continue throughout the whole length, but towards the distal end of the nucleus the thread is seen to branch into two single threads. I interpret this branching thread as one in which the parallel conjugation has not yet been completed. Another instance of the same kind may be seen in figure 31, which represents a stage somewhat more advanced than that of figure 30. These appearances lead me to believe that conjugation begins at the side of the nucleus corresponding to the proximal ends of the leptotene threads, and proceeds gradually toward their distal ends. It is further evident from these figures that conjugation is

not a simultaneous process for all the chromosome-pairs, but that it is a gradual process, some conjugating earlier than others. Just how the members of the different pairs are enabled to select their mates is a very puzzling question, but probably the stretching and orientation of the threads as shown in figure 29 might facilitate this process. That some of the pairs conjugate quite early, is shown in figure 30, where it may be seen that in selected pair *B* conjugation is complete. In figure 27, which is of a very much earlier stage, there are to be seen two of the still hazily defined threads lying side-by-side. They are similar enough in their constitution to be regarded as the two members of a pair, and it would not be surprising if conjugation should begin at a stage as early as this.

As an additional detail it should be pointed out that the bead-like granules which are strung along the threads of the leptotene and zygotene stages are not always of exactly equal size in the two conjugating elements. In figure 58 (Plate **5**) the example of chromosome-pair *B* well illustrates the disparity in size between the two members of some of the pairs of granules. This condition may well answer the criticisms of those who hold that the accuracy with which the granules are paired could be accounted for only on the assumption that they arose by a splitting of single granules into equal parts. I am able to show in this case that the members of each pair of granules are not always of equal size.

3. *The pachytene stages.*— Figures 32 and 33 (Plate **3**) are of early pachytene stages. It sometimes happens that even at such stages there may remain one or two pairs of threads that are not fully conjugated, though I have not added a drawing of such a condition. In the case of some of the pachytene threads of figure 32, complete loops have been formed, both ends being attached at the polar region. The formation of such loops is not necessarily the rule, however, as has been indicated already in connection with the spireme loops of the selected chromosome-pairs (fig. 56–61, Plate **5**). In figure 33 a scattered arrangement of the polar granules is to be seen, though they have coalesced to form several composite granules. Figure 34, of a later pachytene, exhibits one of the large composite granules. Figures 35–37 indicate how the composite granules break up into their component polar granules. A comparison of the examples of chromosome-pair *B* in figures 30 and 35 will indicate the extent of the process of gradual shortening which takes place during the pachytene stages. It will be noticed that the line of separation between the threads which have conjugated (*i. e.*, the primary longitudinal split) remains visible throughout the pachytene stages.

C. The Individuality of the Chromosomes.

a. The selected Chromosome Pairs.

The method adopted in the study of the subject of synapsis — that of following the history of individual chromosome-pairs — has naturally led to a consideration of the subject of the individuality of the chromosomes, that is, their persistence as morphological entities through all the stages of nuclear activity. I have already attempted to demonstrate that each of the chromosomes of the last spermatogonial division gives rise to a single leptotene thread and that these single threads conjugate two-by-two in the zygotene stage. It will be more convincing, however, if we can follow some particular chromosome-pair through these difficult stages.

1. *Chromosome-pair A.*— As the chief characteristic by which the chromosome-pair *A* could be recognized in the pachytene and later stages of the first spermatocytes, I have already described its great density and staining capacity. If there is a persistence of individual chromosomes from the spermatogonia to the spermatocytes, we should expect to find in the former a pair of chromosomes exhibiting the same peculiarities that the pair did in the later generation. Such a pair can, indeed, be found in the telophases not only of the last spermatogonial division but of the earlier spermatogonia as well. Figures 66 and 67 (Plate **6**) show such pairs of chromosomes more deeply stained than their fellows. Figure 66 shows one of the earlier generations of spermatogonia, as is indicated by the vesicular condition of the accessory chromosome, and figure 67 represents a telophase of the last spermatogonial division, as is shown by the condensed accessory at this stage.

It is difficult to follow all the changes that these chromosomes undergo in their transformation into pachytene threads, but I believe that most of the stages are represented in the series of drawings, figures 67–78. Figure 67 corresponds to a stage midway between those shown in figures 21 and 23 (Plate **2**). Figure 68 is of a stage corresponding very closely to that in figure 25 (Plate **3**). In figures 68 and 25, two bands or "blocs" of chromatin can be seen which are more deeply stained than the other chromatin-blocs. The accessory chromosome is distinguishable by its characteristic density and its position at the periphery of the nucleus. The polar granules are also distinguishable. The chromatin in these darker blocs (*A* in both

figures 67 and 68) shows a more or less well-defined spiral condition. This spiral is better shown in figure 69, where it is more unravelled. Very soon after the process of uncoiling gives rise to the leptotene threads, stages in conjugation may be seen. Figure 70 shows an early leptotene stage with two threads which stain more deeply than the others, having conjugated as far as they can be traced in this particular section. I think we may identify these denser threads as the members of the chromosome-pair *A*.

The two sides of figure 70 are drawn differently. The left side is diagrammatic and is intended to represent the apparent entanglement of the leptotene threads. On the right side an attempt has been made to follow individual threads. Careful study makes it evident that the threads, instead of anastomosing, as they appear to do when one makes only a superficial examination of them, are really continuous and distinct for certain distances. The difficulty in following individual threads is due to the fact that after the early leptotene stage the chromatin collects into chromomeres, which are strung along a linin fiber, so fine and stainless in some places that it is scarcely traceable. When two such fibers cross each other in close proximity it is sometimes almost impossible to trace the independent courses of the two in the region of the apparent intersection.

There is less difficulty, however, in tracing the threads of *A*. At the stage shown in figure 71 — which corresponds with that in figure 29 (Plate **3**) the threads are very fine and well oriented. In this nucleus there can be seen a loop of heavier threads (*A*), which have the appearance of being two, loosely wrapped around each other. The accessory, as shown at *X*, also forms a heavy spireme loop at this stage, but it is so much heavier than the one described that there can be no confusion between the two. The deeply staining loop of interlaced threads I interpret to be the spireme of the chromosome-pair *A*. In figure 73 is shown an *A*-spireme which has not completed its conjugation. It will be noticed that of the other threads in this nucleus some are double and some are single; and, furthermore, that the double ones are twice the width of the single ones. In figure 74 the spiremes of the pair *A* have completely conjugated, though the general appearance of the cell indicates that the stage is no further advanced than that shown in figure 73. Figures 75–78 (Plate **7**) show the pair *A* in various stages of conjugation at stages closely corresponding to those shown in figures 73 and 74.

I have already traced the pair *A* from the pachytene stage to the metaphase of the first spermatocyte division, so that it now remains to

examine only the stages following that division. Figure 80 (Plate **7**) shows a telophase of the first spermatocyte division as seen when looking from the equator toward the centrosome. There are eleven dyads here, and since the whole number could easily be counted, the accessory is not present. One of these dyads is more deeply stained than the others, and, judging from its size relations, I think we may identify this dyad as one from chromosome-pair *A*. This conclusion receives still stronger support from figures 81 *et seq.* Figure 81 is of a stage slightly later than the one in figure 80, and here we can see the dyad *A* in addition to the accessory dyad, which is less deeply stained than the others and is surrounded by a well-defined clear space, as indicated by the dotted line. In figure 82 is drawn a telophase in which the dyad *A* is shown in both the daughter cells. From these figures (80–82) it is apparent that this element cannot be confused with the accessory at these stages. In figures 83 and 84, however, it is less easy to distinguish between them. But a long and careful study has convinced me that the accessory, having early passed through a stage of greatest diffusion, soon becomes condensed, while the other dyads are undergoing dissolution. Dyad *A*, on the other hand, at first remains more condensed than the others and then gradually becomes diffused like them. Figure 83 shows an early interkinesis stage in which the large accessory dyad (*X*) is more condensed than that shown in figure 81, but where dyad *A* is still more dense. In figure 84, which is of a stage not much further advanced, the accessory is seen to be the most condensed dyad (*X*), whereas *A* has gone far toward its stage of diffusion corresponding to that of the other chromosomes. That the accessory remains condensed throughout interkinesis is further shown in figures 46 and 48 (Plate **4**). Figure 48 further shows that in the prophase of the second spermatocyte the *A* dyad condenses earlier than any other dyads except that of the accessory.

It was impossible to trace the *A* dyad into the metaphase of the secondary spermatocyte, but in the telophase it may again be recognized by its characteristic deeper staining and by its size relations. In figure 55 (Plate **5**), which is a polar view of such a telophase, three deeply staining chromatic masses are shown. The larger one (*X*) is probably the accessory, the next in size, the monad of *A*, and the smallest, a monad of *B* (p. 79). Figure 85 (Plate **7**) shows a somewhat later telophase, in which diffusion has progressed a little beyond that seen in figure 55. About the same relative staining qualities and relative sizes are seen as in figure 55. The accessory appears in only half of the secondary spermatocytes and spermatids, however, and

figure 86 is of a pair of spermatids in which the accessory does not occur. The diffusion process has here proceeded beyond that shown in figure 85, but the two more deeply staining masses, representing the monads of *A* and *B*, can readily be distinguished.

We may on the strength of this evidence say that the chromosome-pair *A* can be traced from the spermatogonia to the spermatids, thus demonstrating a case of morphological identity through all these generations and stages.

2. *Chromosome-pair B.*— If morphological continuity is the general rule, and if the peculiarities of the chromosome-pairs *B* and *C* are distinctive enough, we should be able to trace the latter as we have traced *A*. In many stages, however, these smaller pairs are not so easily recognizable as was the pair *A*, but it has been possible to obtain good evidence for individuality even through them.

I have called attention to a dyad in interkinesis, and a monad in the spermatids, which seem to satisfy requirements for identification as the element *B*. In figures 80 and 83 (Plate **7**), for example, is seen a dyad smaller than *A*, which stains almost as deeply as the latter. An element with similar properties is to be seen in figures 55 (Plate **5**), 85 and 86 (Plate **7**). This element (*B* in the figures) has such size relations when compared with *A* and the smallest element (as seen in figure 80) as we should expect in *B;* when we consider, further, that in the postspireme stages *B* stained more deeply than the majority of the other tetrads, the staining qualities exhibited in these later stages should also furnish a means of identification.

When we look at the spermatogonial telophases of the same individual from which figure 63 was taken, that is, one in which the components of pair *B* are equal, we can readily find a pair of chromosomes that possesses the chief characteristic by which *B* was recognized in the postspireme stages, namely, the presence of a prominent polar granule at each end and a third not far from the middle, though nearer the distal end. Examples of such spermatogonial telophases are shown in figures 87–96 (Plate **8**). A further consideration of these stages is given on page 83.

The study of chromosome-pair *B* in the growth-period has furnished some of the most interesting data on the subject of chromosome individuality that I have secured. An analysis of this pair in its extended condition in the pachytene stages of the first spermatocyte was made for one of the specimens (no. 772) and then comparisons drawn between the conditions in this and those in all the other animals in the series studied.

Figure 58 (Plate 5) shows the element during the zygotene stages — as indicated by the incompletely conjugated pair of threads near the middle of the figure — in a condition of complete parallel association for the two conjugants, but a condition in which the members of the pairs of granules, the chromomeres, are distinct. A close examination of this spireme of *B* discloses a series of chromomeres in addition to, and smaller than, the three already mentioned as characterizing the element.

For convenience in description the more prominent granules or chromomeres will be given separate designations. The five granules which I wish to mention more particularly will be numbered in order from the proximal (no. 1) to the distal end (no. 5, figure 58). I shall also call attention to the two pairs of small granules between numbers 3 and 4 and to the two pairs of still smaller ones between 2 and 3. I should not omit to direct attention to the series of granules between numbers 1 and 2 and between numbers 4 and 5, but detailed consideration of those already mentioned will probably suffice for the purpose in view.

I was at first impressed by the constancy in relative size and position with which some of these granules recurred in different examples of *B* and at different stages in a single individual (no. 772). It then occurred to me to compare the same element at about the same stage for all the thirteen animals from which material was available. Figure 97 (Plate 8) is the result, each of the separate drawings having been taken from a different animal. The constancy with which the minute details of size and arrangement of the parts of this pair were repeated in all of the individuals was surprising. Not only are the five more prominent chromomeres repeated in approximately the same relative sizes and positions,— as shown in figure 97, where corresponding granules are connected by dotted lines,— but there is likewise a striking correspondence in the more minute details. For example, the segment between the granules numbered 3 and 4 always contains two pairs of granules of about the same relative size, though they vary somewhat in relative position. On the other hand, the segment between 2 and 3 is characterized by the entire absence of any prominent granules. In some cases, however, as in *f*, *i*, and *k*, figure 97, granules can be made out in this segment, and when this is possible there are always two pairs of very small ones in the same relative positions.

It is true that there are some variations in the appearances of the segments between granules numbered 1 and 2 and between 4 and 5, as well as differences in the actual size of the numbered granules.

These variations may be due to one or more of several causes: — (1) Differences arise on account of slightly different reactions to the fixatives and stains. (2) There is a tendency for adjacent granules to fuse, thus causing apparent variations in number and relative size. (3) There is a slight difference in appearance at different stages. (4) The different positions assumed by the element with reference to the optical axis of the microscope may account for some variation in appearance. (5) Some individual variation from animal to animal might be expected.

It will be noticed that the distal granule (no. 5) is single in all the individuals except those represented at *b* and *c*, where it is double. This is in accordance with the statement previously made (page 70) that chromosome-pair *B* is unequal in eleven and equal in only two of the thirteen animals studied. It will also be noticed that the granules at the proximal end (no. 1) frequently become associated with other polar granules in a composite granule (*a*, *h*, *i*, *j*, *l*, *m*, fig. 97), and that with one exception (*f*) the distal end is free. The formation of composite granules is a characteristic feature of this material, as already noted on page 64.

One of the granules of the proximal pair (no. 1) of individual *f*, figure 97, is seen to be enlarged and less deeply stained than its mate. Another example may be seen at *k*. I believe this to be an example of a modification similar to that described in connection with the distal granules of the pair *A* (p. 66). In *B*, this condition appears with much less frequency, for in a count of 84 cases taken at random from one individual only 14 ($16\frac{2}{3}\%$) had one of the granules in the expanded condition. This modification may persist into the tetrad stages, as was the case with *A*. No case was found in which both granules were expanded.

In order to test the variability of the details of constitution of the element *B* in a single animal, a study was undertaken with this object in view. Sixteen drawings (fig. 98, *a–p*) were made of examples taken at random from a single slide. Comparison shows about the same degree of constancy in the composition of the elements here as in the set from different animals. Some of the variations may be pointed out. For example, the relative lengths of the segments 1–4 and 4–5 in example *a*, figure 98, are somewhat different from those in example *h*. I think we may assume that the spireme threads possess some elasticity and that the variation in arrangement, association, and position of the several segments of the spireme may frequently bring about stresses which may stretch some of the threads or parts of threads to a

greater or less degree. The tendency for adjacent granules to fuse probably accounts for some of the variations to be noticed. If one will compare in order the examples *l*, *m*, *a*, and *c*, (fig. 98) the different steps in the fusion of granule no. 4 with the smaller, yet prominent, granule close to it will be seen. As the threads shorten during the later pachytene and postspireme stages, this coalescence of adjacent granules becomes more noticeable and the individual granules all finally lose their visible identity in the compact metaphase chromosomes.

It will be observed that the members of a pair of granules may also appear to be fused together into a single mass. An example of this is seen in figure 98, *n*, granules 4 and 5. This fusion must be very temporary in character, since it is not the general rule, and since the granules separate again in the postspireme stages, as shown in figure 63 (Plate 6); yet so close an association of these granules apparently offers opportunity for the exchange of chemical substances between them. In the case of the proximal granules (no. 1), the members may not only fuse with each other but, as previously noted, characteristically unite with the polar granules of other chromosome-pairs to form the composite granules. The association is fully as close as in that of any single pair, for frequently all traces of the outline of individual granules is entirely lost, as, for example, in figure 34 (Plate 3). Although the individual granules separate out again in the postspireme stages, if we admit that there is an exchange of chemical substances between members of a single pair of granules, I think we must also assume it for the polar granules of the different chromosome-pairs.

At *j* (fig. 98, Plate 8) may be seen another example of an expanded polar granule, such as has already been mentioned. The possible significance of this peculiarity will be discussed on page 112.

It will be instructive to compare the members of particular pairs of granules. Figure 58 (Plate 5), as already mentioned, represents a zygotene stage. The paired chromatic threads near the middle have just begun to conjugate, while in the case of chromosome-pair *B*, in the left half of the figure, the two conjugating threads have only recently come to lie side-by-side, for the members of the different pairs of granules are yet distinct. This condition fortunately gives us an opportunity to compare the relative sizes of the members of each pair. On examination it will be seen that the members of the pair numbered 4 are not equal in size. This is also true for the pair numbered 3. In the case of number 4, the disparity in size between the two granules is considerable, and it is interesting to observe that this difference in

size can frequently be noticed throughout the pachytene stages. Examples of this may be seen at *b*, *e*, *i*, *l*, and *o* in figure 98 (Plate 8), which are drawn from the same individual as figure 58. Similar conditions are also to be found in other individuals, as will be seen in figure 97, *a*, *c*, *e*, *j*, and *l*.

This pair of chromosomes can be recognized in the spermatogonia by the presence, in the telophase, of the three most prominent granules, those I have numbered 1, 4, and 5 in the pachytene stages. Examples of such telophases are represented in figures 87–96. In two cases, where the chromosomes had become considerably elongated in the general diffusion process of the telophase, I was able to make out granules 2 and 3 also with their characteristic relative positions and sizes. These are shown in figures 95 and 96. Where both chromosomes of the pair are recognizable in the same nucleus, there seems to be in every case a difference in size between the two middle granules (no. 4). This difference is probably directly related to the difference noted in the zygotene stage (fig. 58) and the pachytene stages (figs. 97 and 98).

Thus, aside from finding a striking degree of correspondence in the minute organization of the chromosome-pair *B* for all the individuals studied (in the pachytene stages), it has also been possible to trace the pair through all the stages from the spermatogonia to the spermatid, except in the preleptotene and leptotene stages. Figures 30 (Plate 3) and 58 (Plate 5) show that conjugation is completed at a relatively early stage in the zygotene. This precocious conjugation is possibly facilitated by the relatively small size of this pair. The failure to recognize the pair in the leptotene and immediately preceding stages is probably due to the fact that it has not so great a differential staining capacity as has pair *A*, and to the lack of sufficiently long continued study with this object in view.

A further peculiarity of chromosome-pair *B* may be seen upon an examination of figures 99 and 100 (Plate 9). There it will be seen that one end of the tetrad has a peculiar roughened or brush-like appearance, to which McClung ('14) has already called attention. It will be noticed in the same drawings that the accessory chromosome also presents a similar appearance. Furthermore, a like condition is to be seen at the longer end of *C*, as shown in figure 100, and at the end of some of the other autosomes, as seen in figure 99. The roughened contour of the accessory in both metaphase and anaphase of the first spermatocyte division was noted for Phrynotettix by Miss Pinney ('08), and has been described for other species of Orthoptera,

for example by Davis ('08) for Dissosteira and Stenobothrus, and McClung ('14) for various Acrididae. But no one, so far as I am aware, has described such a condition for any of the autosomes. Figure 99 is from a slide that had been treated with Heidenhain's iron-haematoxylin stain, but the destaining process had been carried farther than in most of the other slides. Figure 100 is from another individual, the slides of which had been stained by Flemming's tricolor method, but had not been excessively differentiated. It will be noted that the autosomes in this figure do not exhibit the roughened synaptic ends that are seen in figure 99. It seems probable, therefore, that differences in the staining process may have much to do with the appearance or non-appearance of the roughened condition. In heavily stained slides even the accessory, as well as the tetrads *B* and *C*, may appear with a smooth contour. In this connection, I may call attention to these several points: — (1) Tetrad *B* is unequal in both the cases figured and the roughened end corresponds to the large distal granule on the larger conjugant (see fig. 64, Plate 6). (2) Tetrad *C* is likewise unequal and the roughened end also corresponds with the large distal granule at the end of the larger of the two components (see fig. 65). (3) The polar granules usually occur at the proximal end, *i. e.* the end to which the spindle-fibers attach, and therefore the roughened tips of the autosomes in figure 99 probably correspond to the polar granules of these elements. (4) The accessory chromosome and the polar granules have the common property of remaining condensed while the rest of the chromatin is diffuse, as well as the common property exhibited in these two figures (99 and 100, Plate 9). The suggestion therefore offers itself that there may be some common physical or chemical properties underlying the correspondence in behavior between the accessory and the polar granules.

3. *Chromosome-pair C.*— The drawings of chromosome-pair *B* in figure 64 (Plate 6) and those of *C* in figure 65 were made from sections cut from the same testis. An examination of the spermatogonial telophases of this individual revealed the larger members of each of these pairs very well defined, as indicated in figures 101–105 (Plate 9). No attempt was made to recognize the smaller members of these pairs, because they lacked characteristics, other than size, distinctive enough to make recognition certain. With the larger members of these pairs, however, the distinguishing features are so pronounced that I think there can be no doubt about the identification.

I did not attempt to follow these elements through the preleptotene and leptotene stages, but I have no doubt that careful enough study

would enable one to trace them, as was done in the case of chromosome-pair *A*. It is a matter of no small importance, I believe, that each of the "selected" chromosome-pairs has been recognizable by means of one or both its members, in the spermatogonia as well as in the spermatocytes.

On the other hand, when I came to search through the postspireme stages of the other individuals for tetrad *C*, I was able to find the condition shown in figure 65 in only two instances; but a careful study of these stages in the remaining animals of the series revealed, in place of the large unequal type shown in figure 65, two other types, which are shown in figure 107, *c–m*. Figure 106 presents an example of tetrad *B* from each of the thirteen animals from which material was available for study, and figure 107 a similar series of tetrad *C*. The corresponding letters, *a*, *b*, *c*, etc., in the two series represent the same animal. We may therefore, speak of the different animals as *a*, *b*, *c*, etc. Chromosome-pairs *B* and *C* are the smallest in the whole complex and it will be seen from these two series of drawings that, except in *a* and *b*, the pair *C* is the smaller of the two. In *a* and *b*, *C* is slightly larger than *B*, as was determined by numerous comparisons in the metaphases of the first spermatocytes. The difference in quantity of chromatin in these two cases is quite small, however, and differences in shape and behavior were largely depended on for identification.

For convenience in description, we may designate the three types of chromosome-pair *C* as C_1, C_2, and C_3. By C_1 will be indicated the type, previously described, which is represented in figure 65, and at *a* and *b* in figure 107. The type shown in figure 107, *c–h*, may be designated C_2, and that shown in figure 107 at *i–m*, as C_3. Thus it will be seen that (with a possible exception yet to be discussed) of the thirteen animals studied, two exhibited the type C_1, six the type C_2, and the remainder, five, the type C_3.

If now we compare types C_1 and C_2, it will be apparent at once that both members of the pair C_2 resemble the smaller member of C_1. The homology is striking if one notices the polar granules and the pair of granules close to them, both of which appear in about the same relative size and position in all the examples of both types (except *h*). It is therefore not difficult to believe that type C_2 does actually represent a pair of chromosomes homologous to the smaller conjugant in type C_1.

Turning to type C_3, as shown in figure 107, *i–m*, it will be observed that this is quite different from either C_1 or C_2. It represents an unequal pair but the larger member is very different from the larger

one in type C_1. Furthermore the prominent chromomere near the polar granules does not seem to be present, except possibly at *m* (fig. 107). On the other hand the smaller conjugant resembles those in C_2 in size and otherwise except for the prominent granule already mentioned. We might therefore be led to suppose that the smaller component in C_3 is homologous to the smaller one in C_1 and the two small ones in C_2. But if the example at *h* (fig. 107) be regarded, it will be seen that this is a small pair lacking any prominent chromomere near the polar granules, and might therefore be thought to be homologous with the smaller conjugant in type C_3, if it be considered different from those in type C_2. However, even if the somewhat questionable position of example *h*, be disregarded as to homologies, it still must be admitted that we have at least three different types of chromosomes appearing in these examples of tetrad *C*. I may again point out that there is no chance of making a mistake as to the identity of these elements, for the chromosome-pairs *B* and *C* are the smallest pairs in the complex, and the different types of *C* are mutually exclusive, that is, no two of them are ever found in the same animal. I might further add that all the drawings were carefully outlined with a camera lucida and the details filled in so as to represent as accurately as possible the actual conditions as seen in the microscope. The matter of the possible recombination and redistribution of these different types is discussed on page 121.

b. The Accessory Chromosome.

The accessory chromosome has not been made an object of special study here. Since it has been so thoroughly and so frequently described for orthopteran material, it will suffice to give only a brief account of it in this connection. In the first place, it should be stated that the accessory can be recognized as a distinct chromatic individual at practically every stage from the primary spermatogonia to the spermatid. The fact that it forms a large and faintly staining vesicle or "sac" in all the spermagonia except the last, probably accounts for the occasional statement that it can be first recognized in the telophase of the last spermatogonia, where it appears as a condensed mass of chromatin, or a chromatin nucleolus.

Two points deserve to be emphasized: — (1) The accessory, more than the other chromosomes, maintains an exclusive individuality in nearly all stages. However, it sometimes does become associated

with other chromosomes, especially in the growth-period. Here its polar granule may unite with those of the other chromosomes to form a composite granule. (2) Its behavior, while unique in many respects, differs from that of the autosomes in the degree and the chronology, rather than in the kind, of its changes. The autosomes form vesicles in the telophase of the spermatogonia, as Sutton ('00) long ago pointed out, just as does the accessory, but they are not quite so large or persistent as with the latter. In the growth-period the accessory forms a looped spireme, just as the autosomes do (see fig. 71 and 72, Plate 6), but its thread is much more dense and heavily stained than the others. Although it fails to find a mate in synapsis, its behavior is very like that of the autosomes and its spireme loop may occupy the entire circumference of the nucleus. The process of shortening and thickening, which all the chromosomes undergo, occurs very early in the case of the accessory and it passes through most of the growth-period as a rather compact mass of chromatin. In the postspireme stages, at the time when the chromatids separate from each other by the formation of the secondary longitudinal split, the accessory forms a more or less bent or twisted rod, which often shows a longitudinal split. This split must be homologous to the secondary split seen in the autosomes, which divides longitudinally each of the chromosomes united in synapsis. In the anaphase of the first spermatocyte division its halves separate at the distal end, so that it forms a dyad similar in all respects to those of the autosomes, except for its more roughened condition. In fact, the accessory dyad cannot always be distinguished from the others in the late anaphase. In the metaphase of the secondary spermatocytes it divides along with the autosomes and usually is indistinguishable from them. Its behavior may therefore be more nearly parallel to that of the whole series of chromosomes than we are sometimes led to suppose.

c. The Spermatogonial Divisions.

Let us now consider the subject of persistent chromosomal organization from the standpoint of the spermatogonial divisions. Figures 1–20 (Plates 1 and 2) are intended to represent the most important stages included in the cycle of changes from one cell division to the next. In this description no reference will be made to the selected chromosomes, but the general behavior of the chromatin material will be considered. We shall also leave out of account the mechanics

of the division process and concern ourselves chiefly with the fate of the chromosomes after their division and separation has been accomplished.

In my account of the accessory chromosome, I have already mentioned the formation of sacs or vesicles in the telophases of the spermatogonia. In an early telophase, such as is shown in figure 5 (Plate 1), the chromosomes are clumped together in a rather compact mass at the pole of the spindle. But the distal tips of the larger chromosomes may be seen projecting in various directions. Following the clumped condition, stages occur during which the chromosomes begin to expand and to separate from one another. At the same time there is developed about each chromosome a hyaline area, at first small in extent, but gradually enlarging as the chromosomes continue to expand. These conditions are shown in figures 6–9. Figure 6 is a side view and figure 7 a transverse (optical) section of the same stage. Figures 8 and 9 are likewise side view and optical section, respectively, of a later stage. At this later stage it will be seen that a membrane has been formed at the boundary between the hyaline area and the cytoplasm. We are therefore probably dealing with sacs or vesicles similar to those described by Sutton ('00) for Brachystola.

What is the origin of these sacs? Does the hyaline region as it first appears represent material from the cytoplasm, or from the chromosomes, or is it an artifact resulting from the contraction of the chromatin under the influence of the fixative? That it is not an artifact, will be apparent, I believe, from the following considerations: — (1) The chromosomes themselves, at the stages shown in figures 6 and 7, are larger than in the earlier stages represented in figures 3 and 4. (2) The chromosomes continue to expand and the vesicles expand still more rapidly, as will be seen from the later stages (fig. 8 and 9). (3) The hyaline region as seen in figures 6 and 7 appears more highly refractive than the cytoplasm which would not be the case if it were a space produced by shrinkage of the chromatin.

A comparison of the conditions shown in figures 6 and 7 with those shown in figures 10 and 12 will, I believe, show that the expansion of the vesicles has been at the expense of the cytoplasm. The relative volume of the space within the vesicle as compared with the volume of the cytoplasm, is much less in these earlier stages (fig. 6 and 7) than in the later stages (fig. 10 and 11). Further, it will be seen that the expansion of the vesicles is accompanied by: — (1) an increase in the size of the cell-body, (2) a diffusion of the chromatin into a kind of reticulum within the space of each sac, (3) the breaking down of the

vesicular membranes between adjacent vesicles within the group, especially at the polar end, (4) the formation of an irregular nuclear membrane from the outer walls of the vesicles, (5) the apparent anastomosing of the edges of the networks arising from the diffusion of the chromosomes in adjacent vesicles. The walls bounding the original vesicles are still to be seen in figures 8–13, and this is particularly true of the accessory chromosome, the vesicle of which persists till a late prophase.

What can we now say as to the continuity of the individual chromosomes? Let us first follow the changes undergone by the accessory chromosome. Figures 1 and 2 are of metaphases, in which all the chromosomes except the accessory are compact and smooth in outline. This is roughened in outline and seems to have already begun the process of expansion which characterizes its behavior immediately after division. In figure 4 a hyaline area of considerable extent has already been formed about the accessory, and close examination reveals also a narrow hyaline area just beginning to develop around each of the autosomes. By the time the stage shown in figure 6 is reached, the substance of the accessory has become distributed through the entire space of the vesicle which accompanied the formation of the hyaline area. In its distribution within the vesicle, the chromatic substance is more concentrated on the periphery of the sac, than through the central space. The vesicle continues to expand along with the expansion of the nuclear material as a whole, until the stage of greatest diffusion of the autosomes has been reached (fig. 12 and 13). At the stage shown in figures 14a and 14b (Plate **2**) the chromatin has begun to concentrate towards the axes of the sacs, but this process seems to be less advanced in the accessory (*X*, fig. 14b) than in the autosomes. These are the earliest of the prophases. In the later prophases, as shown in figures 17 and 20, the accessory becomes concentrated as a coiled thread running down through the middle of the vesicular space. The wall of the vesicle persists longer than does that of the nucleus as a whole or that of the other autosomes (fig. 20). There can be no question, it seems to me, that the accessory maintains a persistent individuality through all these stages.

If now the changes undergone by the autosomes be followed, we shall find for them also evidences of persistent individuality. I think no one would deny a persistent individuality up to the stages shown in figures 8 and 9 (Plate **1**). In these figures the chromatin has become reticular, but the masses representing individual chromosomes are still quite distinct and surrounded for the most part by the persisting

walls of the vesicles. The method of formation for these vesicles parallels very closely that described for the accessory, the chief difference being that in the case of the accessory the process is much more rapid. In figures 10 and 11 we find the chromatin much diffused and occupying most of the space within the original vesicles. The vesicular walls are no longer visible, however, except on the periphery as an undulating nuclear membrane, and around the accessory. In spite of this fact, the chromatic masses or blocs, each of which has arisen from a single chromosome, are still recognizable as distinct from one another. This is especially well shown in the optical section drawn in figure 11. There are only eighteen masses shown in this section, but the apparent reduction in number need cause no apprehension as to the fate of the other members of the complex. It frequently happens that the chromosomal vesicles do not all lie parallel to each other, so as to be represented in a single transverse section, and some may even assume a position at right angles to the axis of the majority. Such a case is shown in the upper left-hand corner of figure 10. If, now, we examine the stages shown in figures 12 and 13, which are of the period of greatest diffusion that I have been able to find, we may still see, both in optical section (fig. 13) and in side view (fig. 12), the positions of the individual chromosomes represented by a more condensed band or core. In the case of the optical section, nearly the complete number of chromosomes, as represented by these denser masses or cores, can be counted. It is true that there seems to be an anastomosing system of fibrils connecting the adjacent masses, but this need not mean that there has been a loss of chromosome-identity in a common nuclear mass.

An early prophase is represented by figures 14a and 14b (Plate 2), which show the two sections of a single cell. We see at this stage the beginning of the process of chromatin concentration which results, finally, in the formation of the condensed chromosomes ready for the next division. The chromatic material of each chromosome first concentrates near the middle of the region that it occupied in the nucleus in the diffuse condition. There is thus formed a loosely reticulated core (fig. 14a and 14b), out of which there develops a spirally coiled thread, as shown in figures 15a and 15b. The two stages represented in figures 14 and 15 are very close together in time, for they occurred side-by-side in the same cyst. These coiled threads are at first rather small in diameter, but they rapidly thicken and shorten, as indicated in figures 16–20 (Plate 2). During the process of shortening and thickening the outlines of the vesicular walls become more distinct. This is

especially true of the distal pole, as shown in figure 18. It would seem, therefore, that the vesicular membranes first became formed, then largely disappeared, and later reappeared in part. I am inclined to believe that they actually persist to a greater extent than is apparent. There cannot be any doubt, however, that the vesicles do coalesce at the polar end of the nucleus, for there the individual polar granules frequently fuse to form composite granules, such as may be seen in figure 12 (Plate 1) and figures 14, 15, 16, and 19 (Plate 2).

The first indication of the longitudinal split which forecasts the next mitosis was discernible at a stage such as is shown in figure 17. From this stage on to the metaphase, however, the split was clearly visible.

I believe that the evidence here presented furnishes very good grounds for believing that the chromosomes do not lose their individuality in passing through the so-called 'rest-stage' between the two successive cell-divisions.

d. The somatic Nuclei.

Only slight attention has been given to somatic cells in connection with the subject of the individuality of chromosomes, but some points were noted which it seems worth while to record. The connective-tissue nuclei within the follicle always divide by the indirect or mitotic method. The details are similar to those just described for the spermatogonia, except that individual chromosome-vesicles, even for the accessory, are less conspicuous — in fact, in my limited study of these cells I have not recognized the accessory chromosome with certainty. The only evidence of amitosis is a lobulated condition of the resting nuclei; that condition is a very characteristic one, but has no more significance as to amitosis than the lobulated appearance of the spermatogonial nuclei. In the diffused chromatin-stages — telophase, rest-stage, and early prophases — the polar granules appear, coalesce more or less to form composite granules, and separate out again just as they do in the spermatogonia. Furthermore, it is possible to find chromosomes in the telophases that exhibit all the chief characteristics of the "selected" chromosomes. For example, in Plate 9, *B*, figures 108, 109 and 110, are to be seen diffusing chromosomes with the characteristic features of one of the larger members of chromosome-pair *B*. It would seem from this evidence that the same morphological constitution of individual chromosomes persisted even in these somatic cells.

Going outside the follicle, it is of interest to note what appears in the nuclei of the follicular investment. This investment is a thin membrane inclosing the follicle, forming the outer of the two layers composing the follicular wall. In this membrane the nuclei are very much flattened, so that the chromosomes lie nearly all in one plane. Figures 111 and 112 indicate the chromatic conditions in two such nuclei. It is, I believe, a significant fact that the chromatic masses to be found in these nuclei are in number approximately equal to the unreduced number of chromosomes found in the spermatogonia. Exceptions, it is true, occur; adjacent chromosome-masses may become intimately associated, or one individual mass may become divided into partially separated masses. These nuclei are fully differentiated and are destined never to undergo another cell-division. They must gradually lose their functions and will finally "die in their tracks." The different conditions of the chromatin in the different nuclei suggests that the process of senescent degeneration may have already set in. The important fact still remains, that the individual chromosomes have a tendency to remain distinct from each other, even in these highly differentiated nuclei in a period not only of 'rest' but perhaps of senescence.

D. Summary of Observations.

1. The general topographical relations of the different generations of male sexual cells in the testes of *Phrynotettix magnus* are typical for the Acrididae.

2. For purposes of accurately following the history of the changes undergone by the chromosomes from the pachytene stages of the first spermatocyte to the time of mitosis, three individual chromosome-pairs were selected, each of which possessed characteristics by which it could be recognized in all the stages concerned. These three pairs were designated, for convenience, "*A*," "*B*," and "*C*." A study of these three chromosome-pairs showed: — (a) that there is a longitudinal split in the pachytene stages, which persists into the tetrad and later stages (this is called the primary longitudinal split); (b) that a tetrad is formed out of a spireme segment by (1) a separation along the primary split, and (2) the appearance of a secondary longitudinal split along the middle of each of the two parts separated by the primary split.

3. Tetrad "*A*" opens out along the plane of the secondary split,

the proximal ends separating and moving about 90 degrees apart, so that a rod-shaped element is formed the middle of which represents the distal end of the original segment. The rod, thus extended, becomes oriented with its long axis parallel to that of the spindle and it separates in the middle, thus bringing about an equational division. Tetrad "*A*" also forms rings, but these were not traced into the metaphase, and their later behavior is not known.

4. Tetrad "*B*" occurs in one or the other of two forms: either (1) as an equal pair (in two of the thirteen animals), or (2) as an unequal pair (in the other eleven animals). The unequal pair differs from the equal in the absence of a large terminal granule at the distal end of one of its members. Both types show the same behavior, opening out at both ends of the segment so that a cross is formed. The separation along the plane of the primary split is the greater and occurs at the distal end; but the cross becomes so oriented on the spindle that the short arms (*i. e.* the proximal end of the original segment) are attached to the spindle-fibers. Separation in metaphase is therefore along the plane of the secondary split, thus constituting an equational division.

5. Tetrad "*C*" occurs in three forms, designated C_1, C_2 and C_3. C_1 is composed of very unequal elements, the larger of which possesses a relatively very large terminal knob or granule that is not present on the other. C_2 is a pair with equal members each of which appear to be homologous to the smaller member of C_1. C_3 is a pair of unequal elements neither member of which appears to be exactly homologous to the components of C_1 and C_2. The smaller member resembles those of C_2 and may be homologous to them. The larger member is midway in size between the two members of C_1. C_2 and C_3 divide equationally in the first maturation mitosis, but C_1 divides half the time equationally and half the time reductionally in this first division. When dividing reductionally the two unequal dyads follow the law of chance in their distribution with reference to the accessory chromosome, which passes to one pole undivided.

6. Study of the early growth-stages of the first spermatocyte shows that each of the chromosomes of the telophase of the last spermatogonial division forms a long spirally coiled thread, which uncoils and stretches out to form the leptotene threads of the primary spermatocyte. The leptotene threads conjugate side-by-side (parasynapsis) to form the double threads of the pachytene stage.

7. It was possible to recognize the chromosome-pair *A* in the spermatogonia as two separate chromosomes (telophases) and to

trace the pair through all the stages from the spermatogonia to the spermatids, thus constituting a demonstration of a case of continuous identity, or individuality, through these stages. It was also possible to recognize chromosome-pairs *B* and *C* in the spermatogonial telophases as well as in the second spermatocytes and spermatids.

8. In the earlier pachytene stages, chromosome-pair *B* was found to have a definite arrangement of granules or chromomeres, and it was shown that the relative sizes and positions of these chromomeres remained constant for similar stages, not only in different cells of a single individual, but also in all the thirteen animals.

9. The spermatogonial divisions showed that each chromosome forms a sac or vesicle in the earlier telophases, and that it expands and becomes diffused within these vesicles; that, although the vesicles appear to coalesce, there is always a remnant of each chromosome visible in the center of the region occupied by the vesicle, and that in the prophase the chromatin concentrates about this remnant or core and there forms a spirally coiled thread, which develops into a prophase chromosome.

10. Study of somatic cells showed: — (1) that chromosome *B* could be recognized in the connective-tissue cells within the follicle, and (2) that cells of the follicular envelope, which are probably in a state of senescence, still preserved the normal number (23) of chromatic masses.

11. The polar granules are constant features of the organization of the individual chromosomes, as was shown by Pinney ('08); but in some cases (chromosome-pairs *A* and *B*) they may become modified to give rise to expansions which resemble the "vesicles" described by Carothers ('13), as well as the "plasmosomes" of most authors. The polar granules tend to unite into composite granules at all of the diffuse stages of chromatic evolution.

12. The accessory chromosome behaves in the manner that is typical for the Acrididae. It forms a large separate sac or vesicle in the earlier spermatogonial generations and a peripheral compact mass in the telophase of the last spermatogonial division. During the leptotene and zygotene stages it may unravel into a long loop, which in some cases is equal in length to a great circle of the nucleus. In the pachytene stages it reassumes a compact form, but may be attached by its polar granule to the polar granules of other chromosomes and thus become attached to a composite granule. It passes to one pole undivided in the first maturation division but divides in the second.

III. DISCUSSION.

A. Synapsis and the Maturation Divisions.

It is very difficult to separate the subjects of synapsis and the maturation divisions from the subject of chromosome-individuality. Yet for the sake of clearness it seems best to make such an artificial separation. It might also be possible to separate from each other the subjects of synapsis and maturation divisions, but since the two are so intimately related, it seems better to discuss them at the same time.

Anything like a complete review of the literature on the subjects of synapsis and reduction divisions will not be attempted here, in view of the extensive general reviews in the monographs of Grégoire ('05, '10) and Vejdovský ('11–12), and the reviews relating particularly to orthopteran spermatogenesis by Davis ('08) and McClung ('14).

a. Results from Orthoptera.

McClung ('14) has so recently reviewed the literature on Orthoptera dealing with this subject that it will suffice here to summarize briefly the results. The different views may be classified as follows: —

I. Synapsis not considered.
   a. Both maturation divisions reductional.
      1. Wilcox ('94, '96, '97, '01), Caloptenus.
   b. Both maturations equational.
      1. De Sinéty ('01), various Orthoptera.
      2. Granata ('10), Pamphagus.
   c. First division transverse.
      1. Vom Rath ('92, '95), Gryllotalpa.
      2. Farmer and Moore ('05), Periplaneta.
      3. Jordan ('08), Aplopus.

II. Synapsis described or assumed.
   A. Telosynapsis described or assumed.
      a. First maturation division reductional.
         1. Montgomery ('05), Syrbula.
         2. Stevens ('05), Blatta.
         3. Wassilieff ('07), Blatta.
         4. Zweiger ('06), Forficula.

5. Davis ('08), Acrididae and Locustidae.
6. Buchner ('09), Gryllus, Oedipoda.
7. Stevens ('10b), Forficula.
8. Brunelli ('09, '10), Gryllus, Tryxalis.

b. Second maturation division reductional.
1. Sutton ('02, '03), Brachystola.
2. Baumgartner ('04), Gryllus.
3. McClung ('05, '08a, '14), various Orthoptera.
4. Stevens ('05), Stenopalmatus.
5. Nowlin ('08), Melanoplus.
6. Pinney ('08), Phrynotettix.
7. Robertson ('08) Syrbula.
8. Carothers ('13), Acrididae.

B. Parasynapsis assumed or described.

a. First maturation division reductional.
1. Gerard ('09), Stenobothrus.
2. Morse ('09), Blattidae.
3. Stevens ('12a), Ceuthophilus.
4. Robertson ('15), Tettigidae.

b. Both divisions equational.
1. Vejdovský ('11–12), Locustidae.

c. Division neither reductional nor equational.
1. Otte ('07), Locusta.

This classification [1] is interesting from two points of view. In the first place, it indicates the diverse results that have been obtained by the various investigators working on a limited group within which one might reasonably expect to find a high degree of uniformity in chromosomal behavior. In the second place, the results that I have obtained do not come under any of the classes in the above outline. As stated on previous pages, I have shown (1) that the spermatogonial chromosomes develop into the fine leptotene threads, which conjugate by parasynapsis without the conjugants losing their identity, that is, the line of conjugation is visible throughout the growth-period as the 'primary longitudinal split'; (2) that a second longitudinal split at right angles to the first occurs in the early postspireme stages; and (3) that the tetrads become so oriented on the first maturation spindle that the resulting division is equational. Each of the dyads of the second spermatocytes consists of parts of the two original conjugants,

[1] I have omitted reference to some papers which were non-committal on the points under discussion.

and these conjugant-halves become separated in the second maturation mitosis, the result being, therefore, a reductional division. I am thus able to support the careful studies of McClung and his students as to the orientation of the tetrads in the first maturation spindle, where the spindle-fibers become attached at the so-called 'synaptic' or proximal ends, and therefore bring about an equational division. I can likewise support the findings of those investigators who describe parasynapsis. If we accept the view that one of the longitudinal splits is in reality the line of separation between parallel conjugants, we can also accept the observations of De Sinéty ('01) as to the existence of two longitudinal divisions.

McClung and those of his students who have worked on orthopteran material have derived their results from studies confined largely to spermatogonia and the postspireme stages. There is nothing in any of their figures, however, which would be incompatible with parasynapsis. And the figures by Sutton ('02, fig. 5a, 5b, 6 and 7) of early postspireme stages in Brachystola are much more satisfactorily interpreted from the standpoint of a preexisting parasynapsis than from the standpoint of telosynapsis. I may also state that I have recently examined some Brachystola material and am well satisfied that the conditions there are quite comparable to those prevailing in Phrynotettix. McClung in his latest paper ('14) accepts the possibility of parasynapsis, and Robertson, who in 1908 argued for telosynapsis in Syrbula in no uncertain terms, has recently found parasynapsis in the Tettigidae (Robertson, '15).

A glance at the outline of the results of orthopteran studies given above reveals the fact that parasynapsis has relatively few adherents. I believe the failure to recognize this important stage has been due (1) to the general unfavorableness of these synapsis, or lepto-zygotene, stages for the elucidation of the conditions and a consequent failure properly to interpret them, or (2) to attention having been largely confined to the postspireme stages. That a study of the latter stages could allow of quite diverse interpretations, I am keenly aware, for it was not till I undertook to follow the history of individual chromosomes that I was able to arrive at any satisfactory conclusion as to the sequence of events. I am confident that the use of the same method on other material will reveal conditions similar to those that I have described for Phrynotettix.

Where the chromosomes differ among themselves as to shape, as they do in Stenobothrus, another source of confusion is encountered, for very few authors have recognized the fact that chromosomes of

different shape may behave differently in their orientation on the maturation spindles. McClung has recently gone over this matter in a very painstaking way, and I can agree with his conclusion that, in general, the chromosomes with the spindle-fiber attachment terminal, that is, rod-shaped chromosomes, are oriented in the first maturation spindle so as to produce an equational division, while those which have the spindle-fiber attachment non-terminal, that is, at the apex of V-shaped chromosomes, become oriented so as to bring about a reduction at the first division. This general rule is of course violated when the pairs of rods are of unequal length, which usually (Baumgartner, **'11**; Payne, **'12**; Carothers, **'13**; Robertson, **'15**), but not always (C_1, described in this paper), divide reductionally in the first division. Davis (**'08**) sought to establish the behavior of the V-shaped chromosomes of Stenobothrus as the type for the Orthoptera in general. He correctly described the behavior of these chromosomes in the maturations, but fell into error by attempting to make the rod-shaped chromosomes conform to the same type of behavior. He also failed to recognize parasynapsis. I have recently made a study of the conditions in Stenobothrus and may say that I found parasynapsis for both forms of chromosomes, and that the V-shaped chromosomes divide reductionally in the first maturation mitosis, as Davis described, but that the rod-shaped chromosomes divide equationally in the first division, as I found that they did in Phrynotettix.

b. Recent Work on Synapsis.

That parasynapsis has a wide occurrence, is evident from a glance at the cytological literature, especially within recent years. Grégoire in his two admirable monographs (**'05**, **'10**) has reviewed most of the previous literature bearing on the subject of the behavior of the chromosomes in maturation, and has endeavored to find a common type of behavior for both plants and animals. He says (**'10**, p. 384): "Dans un bon nombre d'objets animaux et végétaux, les cinèses de maturation s'accomplissent suivant le type d'une *prérêduction hétérohoméotypique* préparée par une *pseudo-rêduction prophasique par parasyndèse ou zygotènie.*" In this "hètèrohomèotypique" scheme, however, Grégoire has failed to distinguish the difference in behavior between the chromosomes with terminal and those with non-terminal spindle-fiber attachment. Since the publication of Grégoire's later monograph, a considerable number of investigators have reported the existence of parasynapsis.

De Saedeleer ('13) finds in ASCARIS all the typical stages of the growth-period:— leptotene, zygotene, pachytene, and diplotene; he consequently believes that parasynapsis occurs.

Among the CRUSTACEA parasynapsis has been found by McClendon and by Kornhauser for Copepoda and by Fasten for Cambarus. McClendon ('10) found parasynapsis in both the oögenesis and the spermatogenesis of *Pandarus sinuatus*, but could not decide which of the maturation divisions were reductional. Kornhauser ('15) gives a very full account of a careful study of the process of parasynapsis in *Hersilia apodiformis*, thus confirming the earlier results, as to the existence of parasynapsis in Copepoda, of Lerat ('05), Matschek ('09), and McClendon ('10). In this paper he clears up the uncertainty in regard to this group brought about by the unique theories held by Häcker ('92) and his followers. Kornhauser demonstrates very clearly that the so-called 'Querkerbe,' which Häcker and his followers interpreted as the point of end-to-end union, is nothing more than the synaptic point of the chromosomes which have a median or non-terminal spindle-fiber attachment. The Copepoda are thus brought into line with the majority of other forms. Fasten ('14) finds parasynapsis in Cambarus, and although he is dealing with a very large number of chromosomes (the diploid number is about 200), his figures of the leptotene and zygotene stages are quite convincing.

With respect to work on insect material, I have already mentioned that on ORTHOPTERA. The results of Stevens are unusual in that she has described telosynapsis for Blatta ('05), Stenopalmatus ('05), and Forficula ('10b), while in Ceuthophilus ('12a) she found parasynapsis. In the last mentioned article she says (p. 227) "I should not be surprised if the range of variation should prove to extend from (a) cases where there is nothing that could be called conjugation, but merely such a pairing without contact even, as will secure segregation of homologous maternal and paternal chromosomes to different daughter cells, through (b) an intermediate condition of telosynapsis and less intimate parasynapsis, to (c) cases where homologous chromosomes are so completely fused in parasynapsis that it is impossible to tell whether the resulting chromosomes which are segregated in mitosis are identical with those that went into synapsis or not." It may be that more intensive studies will reveal greater uniformity of behavior than Stevens advocated.

Payne ('14), in a brief description of tetrad formation in Forficula sp., reaches only tentative explanations and conclusions. He finds a variable number of chromosomes in the two maturation divisions and suggests that this might be accounted for by supposing that some of

the spermatogonial chromosomes had failed to pair. He describes two methods of ring-formation. The correctness of his conclusions as to the succession of stages in some of his series might be questioned on the ground that they are not different stages of the same chromosome. The series shown in his figures 2 to 11 probably represents a normal method of ring-formation, viz., by the opening out of a parasynaptic spireme segment along one of the longitudinal splits, with the ends remaining in contact. The series in figures 12 to 16 might also easily be derived from a parasynaptic segment. It is extremely questionable whether the figures in the series 18–20 are arranged by the author in their natural sequence; the reverse order is more likely to be the correct one. His figures 19 to 27 (Plate 2) doubtless represent different shapes of the same chromosome-pair, the so-called "middle granule" serving to identify the element. I would suggest, however, that the stage that he represents in figure 19 may have resulted from an opening out of a parasynaptic segment in the same way that I have described for chromosome-pair *A*, in which case the "middle granules" would be polar granules instead of "middle" ones.

In view of the rather far-reaching conclusions that Robertson ('15) has drawn from his work on the Tettigidae, I would call attention to some differences, as well as similarities, between his work and mine. In the first place, he describes parasynapsis in the early stages of the growth-period, as I have done, but in the postspireme stages he assumes that the conjugants separate along the plane of conjugation (primary longitudinal split), the separation beginning at the proximal end. He shows in the metaphase of the first spermatocyte most of the chromosomes as elongated rods with appearance and orientation similar to that seen for my tetrad *A*. It will be remembered that in the latter case the separation from the proximal end of the spireme segment toward the distal end is not along the plane of the primary split, but along the plane of the secondary split. I believe that Robertson may have overlooked a similar behavior in the chromosomes of his material. Curiously enough the unequal elements that he describes are very similar to the unequal type of chromosome *B* and of chromosome *C*, in Phrynotettix. I have shown that the behavior of the chromatids in *B* and *C* is similar to that in *A*. And that in the cases in which *C* divides reductionally in the first division the spindle-fiber attachment is necessarily shifted to the distal ends. I believe that such a condition probably occurs in the unequal tetrads described by Robertson, and if so, theoretical explanations, as to how the elements came to be related to each other in the way they are,

would be unnecessary. Robertson says he believes that the unequal tetrad in *Tettigidea parvipennis* has arisen by a loss at the distal end. And he finds in other individuals an homologous pair each member of which is equivalent to the larger member of the unequal pair. This is just the condition that is presented by *B* in my material. And, furthermore, by analysis of the pachytene and postspireme stages, I am able to say just what part has been lost.

Another striking analogy between my observations and those of Robertson occurs in connection with the larger unequal pair that he has found in *Acridium granulatus*. He finds in some individuals a small equal pair and in two individuals an homologous unequal pair, the smaller component of which corresponds to either of the two elements of the equal pair. This, again, is precisely the relationship between types C_1 and C_2 in Phrynotettix. Here, too, we both failed to find the other possible combination, namely, that of a pair of the larger conjugants. These striking similarities lead me to think that the elements described by Robertson may be explained in the same way that I have explained them in Phrynotettix. If such be the case, then the various assumptions as to doubling and "sesquivalent" chromosomes will be unnecessary. I believe, further, that without a doubt the unequal tetrads described by Robertson do divide reductionally in the first maturation division, but that the spindle-fiber attaches at the distal and not at the proximal end; and, furthermore, that there is a very good chance that the other chromosomes may behave as does chromosome-pair *A* in Phrynotettix, and therefore divide equationally, as it does.

Of recent works on HEMIPTERA, the most interesting from the standpoint of synapsis are the papers by Montgomery, Wilson, and Kornhauser. Montgomery ('11) advocated telosynapsis for many years, but in this late paper, in which he described the spermatogenesis of Euschistus, he concludes that pairing is by a process of parasynapsis. I believe it to be a highly significant fact that Montgomery, at the end of his very active career as a cytologist, and with his wide experience back of him, should reverse his former position on the subject of synapsis and should find parasynapsis in this insect, which he had studied and reported on at an earlier date ('01). He says (p. 743): "In the growth period through the pachytene stage there is no longitudinal splitting, for what I had previously ('01) interpreted as such, I now find to be the line of conjugation.... Frequently at certain points along a geminus the chromatin granules appear accurately paired. But this does not appear until a rather advanced stage of the

strepsinema and is by no means regular".... Further on (p. 753) he says: — "During the past year I have also convinced myself of the occurrence of parasyndesis in Plethodon, such as Janssens had described for this object and the Schreiners for Salamandra."

Wilson ('12), in his critical study of the subject, first states the questions that he believes must be answered in connection with synapsis and then gives his reasons for believing in the wide occurrence of parasynapsis. He regards the following questions as still awaiting a satisfactory answer: — "1. Is synapsis a fact? Do the chromatic elements actually conjugate or otherwise become associated two-by-two? 2. Admitting the fact of synapsis, are the conjugating elements chromosomes, and are they individually identical with those of the last diploid or premeiotic division? 3. Do they conjugate side-by-side (parasynapsis, parasyndesis), or end-to-end (telosynapsis, telosyndesis), or in both ways? 4. Does synapsis lead to a partial or complete fusion of the conjugating elements to form 'zygosomes' or 'mixochromosomes,' or are they subsequently disjoined by a reduction division?"

Wilson finds his own material (hemipteran) not altogether favorable for a solution of the problems enumerated, but has been able to study the preparations of Tomopteris, supplied by the Schreiners, and of Batrachoseps supplied by Janssens. He studied also some orthopteran material, including Phrynotettix, secured from McClung. After a study of Tomopteris and Batrachoseps he says (p. 384): "Through the study of Batrachoseps and Tomopteris I have finally been convinced — for the first time, I must confess, as far as the autosomes are concerned — (1) that synapsis, or the conjugation of chromosomes two-by-two, is a fact, and (2) that in these animals (perhaps also in the Orthoptera) the conjugation is a side-by-side union, or parasynapsis." And again (p. 399), "The few observations I have been able to make on McClung's preparations of Achurum, Phrynotettix, and Mermiria....lead me to the impression that a side-by-side union of leptotène threads takes place here also."

Browne ('13), from a comparative study of the spermatogenesis of three species of Notonecta, regards the evidence, though not absolutely conclusive, as indicating a conjugation by parasynapsis. Kornhauser ('14), as a result of a very careful study of the spermatogenesis of two species of Enchenopa, finds conclusive evidence of a parasynaptic union at the beginning of the growth-period. The evidence for parasynapsis in the Hemiptera is thus seen to be very strong.

Of recent work on the DIPTERA, I may mention that of Stevens and of Taylor on Culex, and that of Metz on Drosophila. Stevens ('10a) finds parasynapsis in Culex, not merely in the growth-period of spermatogenesis, but among other kinds of cells. She says ('10a, p. 208): — "That parasynapsis occurs immediately after the last oögonial mitosis is certain and it is equally certain that the chromosomes are similarly paired in earlier generations of the oögonia," Again (p. 209): — "In Culex it is quite certain that parasynapsis occurs in each cell generation of the germ cells in the telophase," and (p. 212), "It is interesting to find in Culex a clear case of parasynapsis in oögonia, oöcytes, spermatogonia, and spermatocyte prophases and then to see the same chromosome pairs appearing in the first maturation metakinesis as though united end-to-end." (It is probable that she has overlooked stages in the postspireme showing the changes undergone by a parasynaptic spireme segment in its transformation into a metaphase tetrad). Miss Stevens found six to be the somatic number of chromosomes in Culex, the reduced number being three. The side-by-side pairing of the chromosomes in nearly all generations of cells studied, gave the appearance of a reduced number in many situations where it would not have been suspected. Taylor ('14) states that she found in *Culex pipiens* only three chromosomes in all the stages that she studied, *i. e.* in both the somatic and germ-cells of both sexes. This is a very surprising result, but an explanation may perhaps be found in the conditions observed by Miss Stevens, namely, the tendency for the chromosomes in all kinds of cells to pair between mitoses. A poor fixation might easily prevent one from recognizing the double nature of a closely adhering pair of chromosomes. Besides, Miss Taylor found some cells with six chromosomes, and shows figures of some others with more than three. It would seem more reasonable, then, to regard the prevalence of the reduced number found by Miss Taylor as the result of the constantly recurring tendency of the chromosomes to unite side-by-side between successive mitoses, and possibly to poor fixation.

The results of Metz ('14) on Drosophila are interesting in this connection, for he reports conditions in these flies similar to those found in the mosquito. In this he confirms the earlier results of Stevens ('08). He finds (p. 55) that: — "The chromosomes not only exhibit a close association in pairs at nearly all times, but that before every cell division the members of each pair become so intimately united that they may be said actually to conjugate. Each pair, with the possible exception of the sex chromosomes, goes through what

amounts to a synapsis in every cell division, so that in many cases the figures closely resemble the haploid groups. Apparently this takes place especially in early prophase, but a second conjugation may occur during metaphases, just a short time before division. In the second, or metaphase, conjugation, at least, it is worthy of note that the union is unquestionably a side-by-side or parasynaptic one." Thus we find parasynapsis in a greatly exaggerated form in these examples from the Diptera.

Of recent studies on VERTEBRATA, we may note those of Snook and Long on an amphibian, of Jordan on an opossum, and of Wodsedalek on the pig.

Snook and Long ('14) find the same kind of evidence for parasynapsis that has been presented for Batrachoseps by Janssens ('05), for Salamandra by the Schreiners ('07), and by Wilson ('12) as quoted at p. 102. This evidence, together with that announced by Montgomery ('11) for Plethodon, forms a series of observations which renders very probable a general occurrence of parasynapsis among amphibians.

Jordan ('11) describes in the spermatogenesis of an opossum what he considers evidence for telosynapsis. His figures, however, are far from convincing on this point, since they could as readily be interpreted in favor of parasynapsis as telosynapsis.

Wodsedalek ('13), in his studies of the spermatogenesis of the pig, is unable to find conclusive evidence on the subject of synapsis. He says (p. 13), however, that in the synezesis stage, "The thin threads become arranged in a very much tangled mass of loops, which later appear in about half the original number and twice as thick." Inasmuch as these phenomena accompany every case of demonstrated parasynapsis, the evidence seems to favor the occurrence of this mode of conjugation in this case.

In conclusion, I think it must be admitted that there is abundant evidence for a widespread occurrence of parasynapsis, especially as shown by the most recent investigations. While a majority of the authors who have worked on orthopteran material have reported telosynapsis, I believe there is some chance that many of them were mistaken, or that a more careful analysis of the critical stages would have given a different result. Whether we accept the hypothesis of Stevens, that all degrees of synapsis occur, or the idea of Grégoire, that parasynapsis is an almost universal phenomenon, we must at all events admit that the most careful of the recent investigations indicate that the latter condition is widespread throughout the animal kingdom.

As to which of the two maturation divisions is equational and which is reductional, no absolute rule can be laid down. The evidence, however, points to the probability that generally chromosomes with terminal spindle-fiber attachment are not separated from each other until the second division, while those that have a non-terminal attachment are separated in the first, and that consequently in the former the reduction occurs at the second division, in the latter at the first division.

B. Individuality.

The theory of individuality was early championed by Van Beneden ('83), Rabl ('85), and Boveri ('88). In more recent years the theory has been supported by many writers, who have accepted as substantial evidence in its favor the constancy in the number, size, and shape of the chromosomes reappearing in the mitotic spindle of any one species of animal or plant. On the other hand, some eminent zoölogists have attacked the theory on the ground that the individual chromosomes cannot be traced through the so-called "rest" period between mitoses. It will, therefore, be convenient to discuss the two topics: — (a) constancy in metaphase chromosomes, and (b) persistent organization of chromosomes.

a. Constancy of Metaphase Chromosomes.

1. *Constancy in number.*— The constancy in number of chromosomes for any species is among the most commonplace of cytological observations. It will therefore be unnecessary to make any extensive references to the literature. Some exceptions to the general rule occur, however, and should receive attention. Supernumerary chromosomes have been reported from time to time, and have been studied especially by Stevens and Wilson. Wilson ('09) found in Metapodius variations in chromosome-number from 21 to 26, though the number for each individual animal was constant. The number of chromosomes was dependent neither on sex, nor locality of habitat, nor was it correlated with constant differences of size or of visible structures in the adults. But the variation affects only particular classes of chromosomes (the small idiochromosomes) and all exhibit the same behavior. Furthermore, Wilson found a few cases of mitoses in which both members of a pair of small chromosome were going to

the same pole. Using this as a basis, he was able to find satisfactory explanation of the variation in number, and one which served to support the theory of the individuality of the chromosomes. Stevens ('12b) found in Diabrotica supernumerary chromosomes varying in number from 1 to 5, and believed that they had their origin in transverse and longitudinal divisions of the X-chromosome, which normally divides only longitudinally. These anomalies can therefore be explained on the basis of some unusual method of distribution of the chromosomes in mitosis; the fact that such extra chromosomes persist in all the cells of the animal in which they are found is a striking bit of evidence in favor of the belief that they maintain their individuality.

Della Valle ('09, '12) has attacked the theory of individuality, declaring that the chromosomes are temporary and variable structures, which form in the prophase and dissolve in the telophase. He thinks their number is the quotient of the quantity of chromatin divided by the average size of the chromosomes. The quantity of chromatin is said to vary with conditions of nutrition, and the number of chromosomes with variations in external conditions. He made counts of chromosomes from cells of the peritoneum of salamander larvae and obtained numbers varying from 19 to 27. Montgomery ('10) points to the following grounds for doubting the accuracy of Della Valle's conclusions: — "1. The chromosomes counted are long, sinuous ribbons, that overlap and interlace, the most difficult kind to count with accuracy. 2. He included in the counts some cells in prophases, where one cannot be certain that all the chromosomes have fully separated. 3. The total number of the chromosomes is so large, about 24, that the chance of error in enumeration is great. It is but fair to conclude that while his technique was excellent, his choice of material was bad, consequently a degree of scepticism might well be maintained toward his results." Della Valle in his latest paper ('12) argues that the chromosomes are variable structures, because he has been able to find transition stages between mitotic and amitotic methods of cell-division in the erythrocytes of young salamanders. It is a well-known fact that amitosis frequently accompanies degeneration, and the figures of Della Valle present strong indications of being those of degenerating cells. It is precisely in degenerating cells that one would look for inconstancy in the behavior of the chromosomes.

2. *Constancy in size and shape.*— It will be convenient to consider the subjects of size and shape together. As to shape, we may distinguish spheres, rods, and V-shaped elements. Spheres are invariably small and may be regarded as short rods. It will be convenient to

include under the term "V-shaped" all the chromosomes which have a non-terminal spindle-fiber attachment. They may be regarded as rods which have become bent at the point of the spindle-fiber attachment. Broadly, therefore, we may look upon all chromosomes as rod-shaped, but it will make description easier to distinguish the types just mentioned.

In attempting to show that chromosomes have a constant size and shape for each species, as well as a constant number, it will be well to call attention to the fact, so clearly stated by McClung ('14), that the point of the spindle-fiber attachment is, as a general rule, constant and therefore one of the indications of a persistent organization for each individual chromosome.

Some groups of animals exhibit a high degree of uniformity in the shape and size of the chromosomes in any species, as for example, among the Crustacea and the Amphibia, while others show a great variety of forms (Orthoptera, mammals). In the groups with diverse shapes and sizes of chromosomes, the striking fact was pointed out by Montgomery ('01) that there are two of each different size. Montgomery reached the logical conclusion that of the two equivalent series existing in each cell, one had been derived from the maternal and the other from the paternal ancestor.

That the same series of sizes and shapes reappears in each cell-generation, is recorded by nearly every observer whose material is favorable enough to admit of such comparisons. The work of McClung ('00, '02, '04, '08b, '14), Sutton ('02, '03), Baumgartner ('04), Nowlin ('08), Pinney ('08), Robertson ('08) on orthopteran material has done much to establish this fundamental feature of individuality. A very interesting series of observations on this point is that of Meek ('12a, '12b) on Stenobothrus. He describes the results of a series of careful measurements of chromosome-dimensions in different generations of cells, and as a result of these observations becomes convinced of the existence of persistent individuality. I may quote some of his conclusions ('12a, p. 24, ff.): — "(1) In all metaphases the relative positions of the chromosomes in the equatorial plate appear to be arbitrary. (2) The rods composing all ordinary chromosomes are cylindrical with rounded ends, and of an uniform and constant diameter, viz., 0.83 micra. In each species eight lengths have been found, and these constitute members of a series in arithmetical progression, of which the difference between consecutive terms is equal to the radius of the rod. The heterotropic chromosome does not belong to this general series, for, although equal in length to the longest rod, its

diameter varies at different points and exceeds 0.83 micra. (3) The rods are indivisible units, and, since each spermatogonial and second spermatocyte chromosome is composed of two, and each primary spermatocyte chromosome of four, their morphological identity is metrically proved. (4) The eight rod-lengths are not the same in any two species; the longest and 5 short chromosomes occur in all, but identity is always established by the two remaining chromosome-rods. (5) The complexes of a species and its variety appear to be identical; differences if existing, are too small to be recognized. (6) The somatic chromosomes are identical with those of the germ cells. (7) The total volume of ordinary chromosomes is the same in spermatogonial and primary spermatocyte metaphases, whereas only half this amount appears in that of the secondary spermatocyte."

For some zoölogists, the fact that for any species the chromosomes reappear in the different cell-generations possessing the same relations as to number, shape, and size is merely an expression of the activities of the cell and signifies nothing as to a persistent individuality of the chromosomes. Fortunately, we are not dependent on this kind of evidence alone, the results of studies of the chromosomes of hybrids, for example, offering still stronger evidence of individuality, as shown by the work of Moenkhaus on hybrids between Fundulus and Menidia, and that on echinoderm hybrids by Baltzer and by Tenent. Moenkhaus ('**04**) could recognize the two sets of chromosomes arising from the pronuclei of the diverse parents by characteristic differences in size. For the first two or three cleavages the two groups tended to remain distinct, but in later cleavages the chromosomes of the two kinds become more and more intermingled, though they are still recognizable by their characteristic sizes. The value of this evidence is obvious, and on this point Moenkhaus says (p. 53): — "As long as the two kinds remain grouped, as during the first two divisions, this fact has little added significance (*i. e.*, that two groups of distinctly different kinds of chromosomes arise), since within each group it would be perfectly possible for the component chromosomes to exchange chromatin granules during the resting period. If, however, as occurs in the later cleavages, the two kinds of chromosomes become mingled, the chromatin granules of both kinds must lie mingled together within the resting nucleus. If from such a nucleus the two kinds of chromosomes again emerge, it amounts almost to a demonstration that the chromatin substance of a given chromosome forms a unit and that unit persists." Baltzer ('**09**) was able to recognize in hybrids between Echinus and Strongylocentrotus certain chromo-

somes which were distinctive of the species from which they were derived. Tennent ('08) found in hybrids between Moira and Arbacia a mixture of two kinds of chromosomes, each variety of which could be distinguished. It is, indeed, difficult to understand how these distinctive chromosomes could recur with such definite characteristics in hybrid embryos, if there is no persistent identity for them.

Variations from the general rule of chromosomal constancy have been recorded from time to time, for example, in the shapes of tetrad chromosomes. In many species there is a tendency for each of the forms of the tetrads to be reproduced in the first spermatocyte metaphase. This is particularly true where the chromosomes are all of a similar shape and size. But even in such cases, there is a variation in the exact contour presented by different chromosomes. It has been made apparent by many investigators, especially by McClung and his students, that the shape of a metaphase tetrad is dependent upon the extent and character of the movement on each other of the constituent chromatids. The work of these authors also shows that homologous chromosomes tend to assume about the same shape in all the cells at corresponding stages of mitosis, but that this condition of similarity has its exceptions. Baumgartner ('04) called attention to the constancy in the number of rings formed among the tetrads of Gryllus, and others have noted similar conditions. However, such a criterion for individuality is not always a safe guide, as was pointed out by Foot and Strobell ('05). Commenting on Baumgartner's paper, they say, in regard to chromosomes in *Allolobophora foetida:*— "We find no *constant* form differences of the chromosomes, the simplest form of the bivalent chromosomes is two rods attached end to end, and these present a variety of shapes, rings, figures 8, crosses, etc., without any regularity or constancy. The free ends of the bivalent chromosomes show a tendency to unite into a ring and in some cases nearly all the eleven chromosomes are rings, and sometimes not a single ring is formed" (footnote, p. 222). A glance at figures 39 and 40 (Plate 4) of this paper will also show a variability in shape of the eleven bivalent chromosomes. In my account of tetrad A, I have shown that this element may or may not form rings, so that this character could not be used as a criterion for identification in the earlier postspireme stages. But in spite of these exceptions, there does exist in many cases a strong tendency for a chromosome to assume the same shape at similar stages in all the cells of an animal, and the exceptions have no significance in relation to the question of a variation in the fundamental organization of the tetrads.

In the case of unequal tetrads, however, variation in shape does have some meaning with reference to chromosomal organization. In the specimens of Phrynotettix which I have studied, the shape of tetrad B in two individuals is fundamentally different from that in the other eleven, because, in the latter, a definite part of one member of the pair is lacking. Similarly, in the case of C_1 and C_2, the difference concerns a definite part of the members of the pairs. But the important thing to be kept in mind is that the organization of each of these tetrads is constant for any individual animal, and such differences as exist between individuals can be readily accounted for.

b. Persistent Organization of Chromosomes.

1. *The selected chromosomes.*— One of the most important conclusions arrived at in the present study relates to the constancy in the finer organization of the chromosomes, both from stage to stage in the same individual, and from one individual to another. This is shown in two ways: — first, by the existence in chromosome-pair B of an architecture that is constant both for any one individual in the various stages in which any architectural condition could be recognized, and likewise for all the individuals studied; secondly, by that of a particular pair of chromosomes (A) recognizable through all the stages from spermatogonia to spermatids, the recognition being made possible by the fact that the chromosomes in question possess properties which are characteristic and constant for all stages.

Both of these selected chromosomes, A and B, tend to stain more deeply than the other autosomes, but this tendency is much more marked in A than in B. If chromosomes possessing similar peculiarities be found in related species, may they not be regarded as homologous to the selected chromosomes A and B of Phrynotettix? I think such homologies could be established. Miss Carothers ('13) shows that the small unequal tetrad in Brachystola is usually associated with the accessory chromosome, and is more intensely stained than the other autosomes. Might it not be possible to analyse this unequal element in Brachystola and determine its relation to the unequal tetrads of Phrynotettix? Since these two genera are closely related, I believe this would be possible. Furthermore, the other unequal tetrads described by Miss Carothers for Arphia and Dissosteira were among the small chromosomes and, on account of the similarity in behavior, might be found homologous to B or C of Phrynotettix.

Miss Nowlin ('08) describes for *Melanoplus bivittatus* a precocious tetrad (no. 11), which always appears in the metaphase as a rod extended parallel to the spindle-axis. Such is also the behavior and form of chromosome *A*. Furthermore, I have examined slides of Melanoplus material and find that it also has a spireme loop that stains more deeply than the others. May not this precocious tetrad of Melanoplus be related to chromosome *A* of Phrynotettix?

Early in the course of my investigation I had the opportunity of looking over some of Dr. McClung's collection of slides of acridian material, and, though a thorough study was not made, I could easily recognize in the pachytene stage of a number of species a spireme loop which stained more deeply than the others. Such loops were found, for example, in species of Aeoloplus, Amphitornis, Arphia, Brachystola, Hadrotettix, Hesperotettix, Hippiscus, Melanoplus, Phaetaliotes, and Stenobothrus. One characteristic of such threads, which, however, is not so marked in Phrynotettix, is a tendency to become associated with the accessory chromosome. This is particularly true of Melanoplus and Stenobothrus, the forms in which Davis ('08) was led by this close association to describe a "double monosome." There can be no question, I think, that these "double monosomes" were merely the accessory chromosome plus one of these deeply stained spireme segments. In view of these facts, the suggestion offers itself, that similarity in the properties and behavior of certain chromosomes in different species may be correlated with their taxonomic relationships. Such correlation was, indeed, seen and discussed some time ago by McClung ('08a). Meek ('12) has already made a comparative study of the sizes of the chromosomes in several species of Stenobothrus, and has reached the conclusion that the five smaller pairs of chromosomes are of the same size in all species, but that the large (V-shaped) pairs differ from one species to another. It still remains to be seen whether or not the chromosomes of different species can be compared on the basis of their details of organization and behavior, as well as size.

2. *The heterochromosomes.*— I believe most observers agree that the heterochromosomes maintain their individuality through the growth-stages of the male germ-cell cycle. On another page (p. 87) I have called attention to the similarity in behavior between the autosomes and the accessory, this has also been noted by many others, so that there is no very good ground for setting up a claim to fundamental distinction between the two kinds. It seems to me, therefore, that if we admit a persistent individuality for the heterochromosomes,

we must at the same time admit a high degree of individuality for the autosomes.

3. *Plasmosomes and nucleoli.*— One of the most puzzling problems that cytologists have to deal with is the behavior and function of the so-called 'plasmosomes' or 'nucleoli.' They apparently exhibit such a variety of reactions to methods of technique, and exhibit such varying relationships to other structures in the cell, that it is almost hopeless even to attempt to classify them. That they play some important rôle in the physiology of the cell, there is not the slightest doubt, but what that rôle is, or what relation they bear to the question of chromosome-individuality, are problems that are far from a solution at the present time.

In my description of the tetrads *A* and *B*, I called attention to a peculiar modification of one of the terminal granules of each. I emphasized the fact that, in the case of tetrad *A*, this modified granule furnished a means of identification for this element. Just what the nature of this modification is, I cannot state definitely, but in the pachytene stage it has the appearance of an expansion of a previously condensed granule, and I have so treated it in my description. The similar condition in *B* appears to arise in the same way, but in this case there seems to be a more definite boundary to the modified granule, which thus resembles the plasmosomes, or "vesicles," described by Carothers ('13). The expanded granule of *A* is usually not homogeneous, some areas within it appearing more dense than others. This condition probably foreshadows that seen in the postspireme stages, where it appears more like a collection of small granules, typically three in number. Miss Carothers described the 'vesicles' that she found as being attached to spireme threads, and in some cases to specific threads. Furthermore, she found that the occurrence of the vesicles extended to several species, and, in some species, through several generations of cells. I am indebted to Dr. McClung and to Miss Carothers for the privilege of looking over some of the material studied by the latter, as well as for the opportunity of studying slides of other species; I can confirm Miss Carothers's observations, and can add that these so-called 'vesicles' are present in nearly every species of grasshopper that I have studied with this object in view.

I believe that the modified granules in Phrynotettix can be homologised with the 'plasmosomes' of other species. I would especially call attention to the fact that these structures are always attached to chromosomes, and that, in Phrynotettix, at least, they always involve a certain part of the chromosome to which they are attached. I

believe we may therefore regard them as being related to the organization of the chromosomes, just as much as the polar granules are. A glance at the literature will show how constantly these structures are found; but no one, except Miss Carothers, so far as I am aware, has suggested that they are always attached to some definite region of a chromosome. It would seem to be worth while for some one to make a study of these structures from this point of view.

The changes in staining capacity which the plasmosomes undergo at different stages raises an interesting question as to what may be their relation to the chromatin of the thread with which they are associated. Do they take up chromatin from the chromatin-thread, thus increasing their own stainability, and give it back again as they lose their power to hold the stain? Do they elaborate chromatin from raw materials in the surrounding cell substance and give it up to the chromatin-thread? Is their chromatic substance different from other chromatin? Or, do they have some other way of becoming for a time chromatic and later non-chromatic? May it not be possible to answer some of these questions by carefully resolving into its chromomeres the chromatin-thread with which they are associated, and comparing the constitution at different stages? I believe this could be done on favorable material. In Phrynotettix, these structures are definitely related to polar granules. Are the polar granules to be classed in the same category as the plasmosomes? Is it possible for a polar granule to become transformed into a plasmosome, and then back into a polar granule again? The last question seems to be answered in the affirmative by the conditions in Phrynotettix. In the case of *B*, for example, one of the proximal granules becomes "expanded" in only about 16% of the cases counted. In becoming expanded it has become like a plasmosome. When it is not expanded, it remains a polar granule. Is it any wonder, then, that the plasmosomes have been called 'variable' and 'uncertain' elements of the nucleus?

Plasmosomes are associated with heterochromosomes, as well as with autosomes. Davis (**'08**), for example, noticed one on the monosome of Stenobothrus, and I have confirmed the observation from slides of my own. Morse (**'09**) found in cockroaches a plasmosome constantly associated with the "chromatin nucleolus" (accessory), and in addition another body in the cytoplasm, which he called a plasmosome. A similar cytoplasmic body, which stains like chromatin, is found in a number of Acrididae. Dederer (**'07**) found a plasmosome associated with the pair of idiochromosomes in Philosamia, and

Blackman ('05) found a plasmosome attached to the accessory chromosome of Scolopendra. A long list might be added to show that plasmosomes have been found associated with particular chromosomes. Many attempts, not altogether successful, have been made to explain the baffling relations to the other cell-structures of such bodies as have been called plasmosomes, nucleoli, chromoplasts, karyospheres, etc., but any future attempts to elucidate these relations must, I believe, be accompanied by a recognition of the relations that these structures bear to the organization of individual chromosomes.

4. *Persistence of chromosomes between mitoses.* It still remains to discuss what may be the nature of the "organization" of the chromosomes in the stages through which the nuclear substance passes from one metakinesis to the next. I shall consider briefly (1) the origin of the nucleus from the chromosomes, and (2) theories of continuity.

(1) *Origin of the nucleus.* In my description of the spermatogonial divisions of Phrynotettix (p. 87–91), I pointed out that each chromosome becomes surrounded, as early as the anaphase, by a hyaline region, that this region expands in the telophase; that the chromatin of each chromosome becomes diffused to a certain extent within its own region; that a membrane becomes formed at the boundary between the hyaline region and the cytoplasm, producing the chromosomic "vesicle"; and that the nuclear membrane consists of the outer walls of the vesicles at the periphery of the nuclear group. I drew the conclusion that the hyaline region was formed at the expense of the cytoplasm and that the material of each chromosome tended to remain within the space of its own vesicle, a core of chromatin being particularly noticeable in the center of this region, and that the prophase chromosome subsequently formed was developed out of the substance of one, and only one, of the previously existing telophase chromosomes. Sutton ('00) was the first to describe the vesicles of the spermatogonia of a grasshopper. Since then, Otte ('07) has seen similar structures in Locusta, and Davis ('08) in several Acrididae; Pinney ('08) has described them for Phrynotettix. Sutton stated that in the earlier stages of nuclear formation, each chromosome produced a separate vesicle, just as I have found for Phrynotettix, but that in later stages, the proximal ends fused together, giving a common nuclear cavity, from which the distal ends of the vesicles, particularly the longer ones, projected out like the fingers of a glove. Sutton interpreted these conditions as lending strong support to the theory of individuality. Otte believed that the individual vesicles remain distinct throughout the whole of the interkinetic phases, and

Pinney reached a similar conclusion. Davis, on the other hand, could recognize only an irregular outline for the nucleus, and did not identify the vesicle of even the monosome with certainty. Gerard ('09) saw the hyaline regions about the telophase chromosomes of Stenobothrus, and stated that the nuclear membrane was formed in connection with them. Since similar conditions have been reported by so many observers, it would seem that these vesicular structures are the result of normal processes and not, as claimed by Vejdovský ('11–12), artifacts.

If we turn to accounts other than those on orthopteran spermatogenesis, we find that the formation of chromosomic vesicles out of individual chromosomes in the telophase is by no means of rare occurrence. Van Beneden ('83) noted in his work on Ascaris that each of the two chromosomes of the female pronucleus often formed a separate 'half-nucleus.' Häcker ('95b) observed that the chromosomes of the early cleavages of *Cyclops brevicornis* formed two groups of "Bläschen," one group from the maternal and another from the paternal pronuclei. Conklin ('02) calls attention to the occurrence of such chromosomic vesicles, and gives the history of the nuclear changes during the cycle of division in Crepidula as follows (p. 45): — "(1) The chromosomes, consisting of chromatin enclosed in a linin sheath, divide and move to the poles of the spindle, where they partially surround the spheres. (2) Here they become vesicular, the interior of the vesicle becoming achromatic, though frequently containing a nucleolus-like body, while the wall remains chromatic. (3) These vesicles continue to enlarge and then unite into the "resting nucleus." The nuclear membrane is composed of the outermost walls of the vesicles, while the inner walls stretch through the nucleus as achromatic partitions. The chromosomal vesicles for the egg and sperm nuclei remain distinct longer than those from the same nucleus .... Such vesicles are found generally, if not universally, in the early division of ova, though they are not usually found in other mitoses." Smallwood ('05) describes similar chromosomic vesicles in the eggs of nudibranchs. He found that during the "rest-pause" between the first and second maturation divisions the chromosomes frequently have distinct vesicles. There may be a single vesicle for all the chromosomes, or a single vesicle for each chromosome; all conditions between these two extremes occur. Medes ('05) in her work on Scutigera found in the second spermatocytes (p. 174) that: — "There is no immediate formation of a nuclear membrane, but each separate chromosome, as it disintegrates, becomes enclosed in a membrane of its

own, thus forming a structure similar to a nucleus but containing only a single chromosome." Kornhauser ('15, p. 408) says concerning the spermatogonia of Hersilia: — "The telophase chromosomes become gradually fainter in outline, and a clear area in the cytoplasm begins to form about them. It is, I believe, the boundary between this clear area and the more reticular cytoplasm which forms the new nuclear membrane." Thus it will be seen that it is quite usual for telophase chromosomes to form individual nuclei, which later fuse to form the whole nucleus, and with Smallwood ('05) we may accept this tendency as an argument for chromosomal individuality.

(2) *Theories of continuity.* Among those who support a theory of continuity, there is not always agreement as to what structures are carried from one cell-generation to the next. It is generally agreed that the chromosomes are composed of at least two substances; the chromatin and the ground substance (linin, plastin). Häcker ('04) formulated the "Successionshypothese," stating that the persisting structures of the chromosomes consisted of the "Grundsubstanz," or achromatic part. Bonnevie ('08a) and others, on the contrary, regard the chromatic substance as the persistent portion and the achromatin as the temporary part of the chromosome.

Vejdovský ('07, '11–12) has evolved a most elaborate theory touching this problem. In his monograph of 1907, he based his conclusions on a study of the ovogenesis and maturation of some annelids. He concludes that the nucleus is derived from the chromosomes and from them alone. He divides the interkinetic stages into two periods; the one during which the nucleus is formed out of the chromosomes he calls "katachromasis," and the one during which the chromosomes are formed out of the nucleus he calls "anachromasis." In his later monograph ('11–12) he analyses these processes still further and attempts to describe in detail the events in the two periods. His conclusions may be briefly stated as follows: — A chromosome is composed of two substances, one a less deeply staining substratum, on the surface of which is the other, the more deeply stainable chromatin. In the early stages of katachromasis, the chromatin differentiates into a spiral thread, or "chromonema," which is coiled about the surface of the substratum. The substratum then dissolves, forming the nuclear sap, or "enchylema." The chromonema further differentiates into a finely coiled chromatic portion, inside of which is a linin core. In this condition, he recognizes the anlage of the chromosome of the succeeding generation. The linin substance of the chromonema is to become the substratum of the future chromosome, and the finely

coiled chromatic portion will become its chromonema. In successive generations, therefore, there is a changing composition of the chromosomes. During each katachromasis, the ground substance of the chromosome dissolves, leaving the chromonema, which becomes differentiated into the two kinds of substance found in the chromosome of the next generation. This is an ingenious theory, to say the least, and carries with it some measure of support for the theory of individuality, inasmuch as each new chromosome is formed out of the substance of a preceding one.

I have found nothing in my studies to support any one of these theories to the exclusion of the others. It is rather surprising, however, that Vejdovský found no indication of the chromosomic vesicles in the spermatogonia of the Orthoptera that he studied and that he regards those seen by others as artifacts. I find little evidence of a chromonema in the telophase of the spermatogonia, and what evidence there is would indicate that the chromatin becomes distributed on the *inner* surface of the vesicular walls, not on the outer surface of an achromatic core. In the telophase of the last spermatogonia, I find a spiral thread forming, but it develops out of the chromatin at the middle of the area occupied by a vesicle. But whether we accept any one of these theories, or reject all of them, there still remain the strongest grounds for believing, as they all indicate, that there is some underlying organization which is in some way perpetuated for each individual chromosome. I am inclined to the belief that this organization involves both chromatic and achromatic substance.

In plant material evidence which indicates a continuity of the chromosomes has not been wanting. Grégoire (**'07**, **'10**) believes that the results of his own investigations and those of others on plants furnish strong support for the individuality theory. Stout (**'12**) has recently added evidence for this belief in his work on *Carex aquatilis*. He says (**'12**, p. 36): — "The chromosomes are present in all resting nuclei as visible units of a definite number. These individual chromosomes can be traced as such through all stages of both somatic and germ-cell divisions, with the exception of the various stages of synapsis (synizesis)." Lee (**'13**) also finds continuity of the chromosomes in plants through the "rest-stage." He believes that the chromosomes of even the metaphase become vacuolated, that this vacuolization increases in the telophase, where, later, a spiral thread is formed out of each chromosome. This spiral thread becomes the prophase chromosome of the succeeding division. He introduces the term "spirophase" to designate the so-called "rest-stage."

There seems to be a great amount of disagreement as to just what constitutes individuality, but I believe that we may class as instances of individuality all cases where it can be shown that the substance of any telophase chromosome gives rise to one and only one prophase chromosome. In that event, any one of the three theories mentioned above would support the theory of individuality. I believe that I have demonstrated individuality for chromosome-pair *A*, and have shown good evidence for it among the other chromosomes of Phrynotettix. Besides, it seems to me much more logical to regard the constant reappearance of the same architectural conditions of a given chromosome as a result of continuity of that architecture in some form or other through all the cell-divisions, than to assume that the organization is entirely destroyed and reestablished between successive mitoses.

C. Chromosomes and Heredity.

Any discussion of the relation of the chromosomes to heredity must deal to a considerable extent with theory and speculation. Yet there are many facts which tend to the belief that the chromosomes are, after all, directly concerned with the transmission of hereditary qualities. A few facts and some theory will be considered in the following paragraphs under the two heads: — (a) Mendelism and maturation, and (b) some experimental evidence.

a. Mendelism and Maturation.

Wilhelm Roux was apparently the first to formulate, in the early eighties, a theory in which an attempt was made to localize the carriers of hereditary qualities in the chromosomes; this was later elaborated by others, especially by Weismann, who postulated a reduction division which has since been identified with one or the other of the maturation divisions. Montgomery ('01) pointed out that the chromosomes of the diploid series occur in pairs, the members of each pair being of the same shape and size. There are thus two similar series of chromosomes. He concluded that one series was derived from the maternal, the other from the paternal ancestor. He concluded further that the members of each pair unite to form the bivalent chromosomes of the first spermatocytes. Boveri ('02) decided from the results of his experiments on dispermic echinoderm

eggs, that the chromosomes were qualitatively different. Sutton ('03) in the following year, explained how the behavior of the chromosomes in maturation could be correlated with the behavior of Mendelian characters. He showed: — (1) that the union of chromosomes of diverse origin into pairs and their subsequent separation in one of the maturation divisions would insure to every gamete one of every kind of chromosome in the series: (2) that if the law of chance were operative in the orientation of the pairs on the maturation spindles, every possible combination of male and female chromosome could result; and (3) that such a recombination according to the law of chance would account for the transmission of Mendelian characters, if the chromosomes retained their individuality and really were the carriers of the qualities.

This work of Sutton has been generally accepted as proving the correlation assumed, but it remained for Carothers ('13) to demonstrate that the law of chance actually does operate in the distribution of the chromosomes in the maturation spindles. In the case of the unequal tetrads described by her, it was shown that either the large or small member of the pair may go to the same pole as the accessory chromosome, which, as usual, was found to go to one pole undivided. Moreover, it was found that the ratio between the two results of distribution was approximately one to one. Robertson ('15) has very recently published some of his work on the Tettigidae, where he has found the same rule to hold for the unequal pairs that were present in his material. The behavior of tetrad C_1 in Phrynotettix agrees with that described by Carothers and Robertson. These cases establish the fact that there really is a distribution of chromosomes in the maturation divisions according to the law of chance.

A further consideration of the cases of unequal tetrads in Orthoptera will show in how far the theoretical possibilities as to chance distribution have been realized. Baumgartner ('11) in reporting his results on *Gryllotalpa borealis* before the American Society of Zoölogists, stated that he found in the first maturation mitosis an unequal pair of chromosomes, of which the larger dyad always went to the same pole as the accessory. Payne ('12) found the same conditions in this species of Gryllotalpa. He regards the large member as possibly associated with the accessory to form a sex-group, similar to the groups in Conorhinus and Fitchia (Payne, '09), or in Thyanta (Wilson, '10), with the exception that in Gryllotalpa the grouping occurs in the first spermatocyte metaphase instead of the second. Payne suggests that the chromosomes instead of following a haphazard

method of distribution in the maturation divisions, may always move the same way, *i. e.*, all the chromosomes brought into the egg may pass into the female-producing sperm. It is extremely doubtful if the last suggestion will prove applicable as a general rule, but the conditions in Gryllotalpa are interesting exceptions to what has been found in the Acrididae and Tettigidae.[1]

Hartmann ('13) describes small chromosomes as dividing unequally in some male germ-cells of Schistocerca. In one first-spermatocyte cell he found two such chromosomes (tetrads) dividing unequally, and he found some cases of unequal division in the secondary spermatocytes. These observations, if correct, would lead one to suspect that he might have been dealing with a condition similar to that in Phrynotettix, except that in the first division, either both the small chromosomes divided sometimes reductionally and sometimes equationally, or, while one of them followed this method, the other always divided reductionally.

Bringing together the results of Baumgartner and Payne for Gryllotalpa, those of Carothers for Acrididae, Robertson for Tettigidae, and my own for Phrynotettix, we may arrange a graded series of conditions beginning with (1) tetrad *B*, in Phrynotettix, which is unequal, but divides equationally in the first division; passing (2) to C_1, which divides with equal frequency either reductionally or equationally in the first division, and when dividing reductionally shows chance distribution with reference to the accessory; thence (3) to the unequal types found by Carothers and Robertson, which always divide reductionally in the first division but show chance distribution, and finally (4) to Gryllotalpa, where division is always unequal in the first spermatocytes, but the larger dyad always accompanies the accessory. Whether this series offers any possible explanation as to the origin of these unequal elements, and their different kinds of behavior, is problematical.

Robertson's work deserves further consideration, because he has found two of the three possible combinations which would be expected out of a random recombination of two unequal elements which conjugate. In the case of Tettigidea, he found the unequal tetrad in

[1] Postscript.— Unfortunately I had overlooked the results reported for *Gryllotalpa vulgaris* by Voinov ('14), who found in the first spermatocyte metaphase an unequal pair of dyads, which separate so that sometimes the larger dyad and sometimes the smaller one goes to the same pole as the accessory chromosome. These results are in accord with those mentioned above for the Acrididae and the Tettigidae and it may be surmised that similar conditions perhaps obtain for *Gryllotalpa borealis* but have so far been overlooked.

only two individuals, all the others showing a pair both members of which were equal to the larger member of the unequal pair. The third possibility, an equal pair, homologous to the smaller of the dyads, was not found. This case is analogous to that of *B* in Phrynotettix.[1] In Acridium, Robertson found two individuals, one a male, the other a female, possessing an unequal pair of chromosomes, whereas all the other individuals studied showed the homologous pair to be equal, both members being equivalent in size to the smaller of the two members of the unequal pair. This case is analogous to those of tetrads C_1 and C_2 in Phrynotettix, where, also, only two combinations, the same two, out of a possible three have been found.

Robertson calls attention to the obvious possibility of a loss of chromatin from the unequal pair in Tettigidea, and suggests that the loss of Mendelian factors could be accounted for in this way. He also suggests that the loss of the distal ends of both the chromosomes, resulting in a pair of small dyads each equivalent to the smaller member of the unequal pair, might result in lethal conditions, or might mean the loss of factors necessary for development. In the case of the unequal pair in Acridium, he assumes that there has been an addition to one member of the smaller pair. If this element is similar to C_1 of Phrynotettix, as it seems to be, then the simpler explanation would be that a part had been lost, just as in the case of the one in Tettigidea. It is curious that in both C_1 and the unequal pair in Acridium, the same combination, *i. e.*, a pair both members of which would be equal to the larger member of the unequal pair, is lacking. I am inclined to believe, if sufficient material were available, that the remaining possible combinations would be found. The matter could, at least, be tested by experiment. It is the hope of the writer to conduct breeding experiments with this object in view.

One further point remains to be considered in relation to chromosome-pair *C*. I have described these tetrads in detail elsewhere (p. 85), but a reference to figure 107 (Plate **9**) will recall that there are three types, which I have designated as C_1, C_2, and C_3. If similar types exist in the female,— Robertson (**'15**) found an unequal pair in a female of Acridium,— and random mating be assumed for the animals possessing the three different types, then one ought to obtain in

[1] Since writing this I have had an opportunity to examine slides from some new Phrynotettix material collected during the summer of 1915 by Miss Carothers of the University of Pennsylvania. In some of the individuals of the new material I have found the expected third type of chromosome-pair B composed of two elements both equivalent to the shorter member of the unequal type.

the offspring all possible combinations of the three kinds of chromosomes. Or, if mating involving any two of the types could be made, there should result all possible combinations between them. On account of the small number of animals available for my study, no conclusions as to whether these conditions are realized in nature could be drawn. The presence of the three types in these few animals, however, strongly suggests the possibility of realization, especially since two of the three possible combinations are realized for the two kinds of chromosome in type C_1. The presence of a third type also suggests that there may exist in this case the mechanism for the transmission of triple allelomorphs.

b. Some experimental Evidence.

The most extensive breeding experiments the results of which tend to show that the chromosomes are concerned in the transmission of hereditary characters are those on Drosophila by Prof. T. H. Morgan and his students. In the course of this work they have dealt with over a hundred unit-characters which show Mendelian inheritance, either in a typical or modified form. In Drosophila, there are four pairs of chromosomes, of which one pair is very small, and one is a pair of heterochromosomes, or "sex-chromosomes." In their behavior in inheritance, the hundred and more characters fall into four groups, each group tending to behave as a unit, just as it would be expected to do in case it were carried by a single pair of chromosomes. Of these groups of characters, one is very small, the others much larger, the largest one being the group of "sex-linked" characters. Naturally the small group of characters has been correlated with the small pair of chromosomes and the group of sex-linked characters with the sex-chromosomes.

But there have been exceptions in the case of many pairs of allelomorphs, especially those that are sex-linked, *i. e.*, cases where factors belonging to a certain group have gone into a mating together, but have not always reappeared together, as they would be expected to do if they were all carried by a single chromosome and that chromosome maintained its individuality. These phenomena have been explained by the so-called "cross-over" hypothesis. In this connection Morgan ('**11**) developed what has been termed the "linear arrangement" hypothesis, which was further elaborated by Sturtevant ('**13**). These authors assume that the factors, or "genes," which represent the

characters, are distributed in a linear series along the length of the chromosomes. Then, invoking the aid of the "chiasmatype" theory of Jannsens (**'09**), they attempt to explain the "cross-overs" by assuming, first, that when two chromosomes conjugate side by side, they may become twisted around each other, and, secondly, that the later separation is along a plane, which cuts across the threads once for every complete twist. Considering the matter in relation to the tetrad stages, it might be imagined that the two threads cross each other, and that at the point of crossing, a weakness of the strands causes them to break and then recombine, forming threads each of which is composed of a part of both the original conjugants.

Judging from his figures, Janssens founded his theory on conditions similar to those shown in my figure 38, *a–d* (Plate **3**). I am quite sure that the evidence in Phrynotettix does not support the idea that the chromatids break and recombine in any of the postspireme stages. On the contrary, I believe that the chromatids maintain as strict an individuality as I have claimed for the chromosomes themselves. And since these tetrad figures are repeated in so many animals, and even in plants, there would seem to be ground for supposing the behavior to be similar in all.

On the basis of this hypothesis, however, Morgan and his pupils have been able to explain the anomalous behavior of the genes which they call the "cross-over" in a very satisfactory way; furthermore, they have been able to use it in connection with the linear-arrangement hypothesis to predict the behavior of any given character, with reference to any other character in the group to which it belongs, provided its behavior in relation to one or two of the characters of the group is known. But there is one point yet to be noted. I have based my criticism of the chiasmatype theory on the conditions as found in spermatogenesis. One of the peculiar facts found in the work on Drosophila is that there is no "crossing-over" in the male. But why should such a phenomenon occur in the female and not in the male? Is it not possible that in the "great growth" period of the oöcyte,— where the tetrads become so much more expanded and diffused than in the male, even seeming to disappear entirely in some cases,— the tetrads might suffer some such changes as those suggested by the experimental results? There is also to be considered the often repeated condition of parasynapsis in Drosophila, as shown by Metz (**'14**), which might offer greater opportunities for such "cross-overs" to occur than are found in other animals.

Whatever else may be said of the results of the experiments on

Drosophila, it must be admitted that they go very far towards establishing a direct relationship between the chromosomes and the transmission of Mendelian characters. Perhaps the most convincing evidence of this kind is that obtained by Bridges. He has found that in certain strains involving sex-linked inheritance, some exceptional females appeared which were like their mothers in every respect, and showed no transmission of sex-linked characters from the father, although such transmission would be expected, since the male sex formula is XY and that of the female is XX. Furthermore, he found that such exceptionally produced females inherit directly from their mother the power of producing like exceptions (about 5%). The explanation advanced by Bridges ('14) was that "the sex-linked genes were borne by the X-chromosomes and that 10% of the eggs of the exceptional females retained both of the X-chromosomes, or conversely lost both to the polar body." This phenomenon was called "non-disjunction." Breeding experiments showed that an X-chromosome gene could not be the cause of the phenomenon, and the prediction was accordingly made that half the daughters of a non-disjunctional female would be found to contain in addition to the two X-chromosomes a supernumerary chromosome which would be a Y. Cytological investigations have shown that approximately one-half of the daughters of a non-disjunctional female do, in fact, contain a supernumerary Y-chromosome, while the remaining half contain only the two X-chromosomes. I may add that through the kindness of Dr. Bridges, I have been able to examine some of his slides and convince myself of the presence of the extra chromosome. This brilliant piece of work makes it very hard to disagree with Bridges's conclusion ('14, p. 109) that, "there can be no doubt that the complete parallelism between the unique behavior of the chromosomes and the behavior of the sex-linked genes and sex in this case means that the sex-linked genes are located in and borne by the sex-chromosomes."

Returning now to a consideration of the linear-arrangement hypothesis, it must be admitted that the theory has attractive possibilities, and up to the present time has stood the test of experimental breeding in Drosophila. It may not be out of place, therefore, to call attention in this connection to the constancy of the granular, or chromomeral organization of the chromosomes of Phrynotettix, particularly in chromosome-pair B. May not this constancy of architecture of the chromosomes have a meaning correlated with that assumed in the linear-arrangement hypothesis? This possibility seems to me to be worthy of further investigation.

D. Summary of Conclusions.

It is believed that the present study of the spermatogenesis of *Phrynotettix magnus* has demonstrated: —

1. That conjugation of the chromosome-pairs is by parasynapsis.
2. That the majority of the bivalent chromosomes divide equationally in the first maturation division.
3. That the chromosomes retain their individuality through the spermatogenic cell-generations.
4. That the so-called 'plasmosomes' take their origin from some definite region (granule) of particular chromosomes, but that they may be variable in occurrence and in extent of development.
5. That in the maturation divisions (*e. g.* chromosome-pair C_1) the law of chance is followed in the distribution of the chromosomes.
6. That each chromosome possesses a definite organization, which is expressed in the constancy of the relative sizes and positions of its chromomeres (as seen, *e. g.*, in chromosome-pair *B*).

In addition, the possibilities are suggested that: — (1) the matter of the behavior of unequal pairs of chromosomes in regard to distribution and recombination may be tested by breeding experiments, (2) the constancy in the arrangement of chromomeres along the length of the chromosome-threads, as described for chromosome-pair *B*, may have a meaning related to that suggested by Morgan's "linear-arrangement" hypothesis, and (3) that in the varying types of chromosome-pair C there may exist a mechanism for the transmission of multiple allelomorphs.

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